THE BULLETIN OF
Mathematical BIOPHYSICS

THE UNIVERSITY OF CHICAGO PRESS · CHICAGO · ILLINOIS

VOLUME 11

1949

PUBLISHED MARCH, JUNE, SEPTEMBER AND DECEMBER, 1949
PRINTED BY THE DENTAN PRINTING COMPANY, COLORADO SPRINGS, COLORADO

THE BULLETIN OF Mathematical BIOPHYSICS

MARCH 1949

TIME VARIABLE OSMOTIC PRESSURES PRODUCED BY COUPLED REACTIONS AS A POSSIBLE CAUSE OF CELL DIVISION—*N. Rashevsky* 1

A NOTE ON THE DIFFUSION DRAG FORCES—*N. Rashevsky* 9

NOTE ON A CASE OF NONLINEAR DIFFUSION—*N. Rashevsky* 15

ON THE VELOCITY OF CONDUCTION IN NERVE FIBERS WITH SALTATORY TRANSMISSION—*H. D. Landahl and R. J. Podolsky* 19

THE STEADY STATE KINETICS OF SOME BIOLOGICAL SYSTEMS: I—*John Z. Hearon* 29

THE GROUP STRUCTURE OF SOME NEURAL NETS—*J. B. Roberts* 51

THE UNIVERSITY OF CHICAGO PRESS · CHICAGO · ILLINOIS

VOLUME 11 · · · · · NUMBER 1

THE BULLETIN OF MATHEMATICAL BIOPHYSICS

EDITED BY N. RASHEVSKY

The Bulletin is devoted to publications of research in Mathematical Biophysics, as described on the inside back cover.

THE BULLETIN is published by the University of Chicago at the University of Chicago Press, 5750 Ellis Avenue, Chicago, Illinois, quarterly, in March, June, September, December. ¶The subscription price is $4.00 per year, the price of single copies is $1.25. Orders for service of less than a full year will be charged at the single-copy rate. ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

THE FOLLOWING are authorized agents:

For the British Empire, except North America, India, and Australasia: The Cambridge University Press, Bentley House, 200 Euston Road, London, N.W. 1. Prices of yearly subscriptions and of single copies may be had on application.

CLAIMS FOR MISSING NUMBERS should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit, and when the reserve stock will permit.

BUSINESS CORRESPONDENCE should be addressed to The University of Chicago Press, Chicago, Ill.

COMMUNICATIONS FOR THE EDITOR and manuscripts should be addressed to N. Rashevsky, Editorial Office of the Bulletin of Mathematical Biophysics, 5822 Drexel Avenue, Chicago, Ill.

IMPORTANT ANNOUNCEMENT

The increase in the output of papers in the field of mathematical biophysics makes it difficult to insure prompt publication without an increase in the size of the journal. Therefore, the Bulletin of Mathematical Biophysics inaugurates the following service:

Upon acceptance of a paper, the Editor will, if necessary, ask the author to shorten the paper to an extent dictated by the requirements of a reasonably prompt publication. The shortening should in no case reduce the paper to a mere abstract. Such a shortened paper will be published within six months or less.

The unabbreviated original manuscript will be kept on file at the editorial office. Any person desiring to avail himself of the complete manuscript may obtain promptly a microfilm copy of the latter, at the cost of 2¢ per page plus a carrying charge of 25¢ and postage, by applying to the Editorial Office, 5822 Drexel Avenue, Chicago, Illinois.

All papers in the Bulletin which have been thus shortened will be marked at the end by the symbol MF followed by a figure indicating the number of double-spaced typewritten pages of the unabbreviated manuscript.

PRINTED BY THE DENTAN PRINTING COMPANY . . . COLORADO SPRINGS, COLORADO

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 11, 1949

TIME VARIABLE OSMOTIC PRESSURES PRODUCED BY COUPLED REACTIONS AS A POSSIBLE CAUSE OF CELL DIVISION

N. RASHEVSKY
COMMITTEE ON MATHEMATICAL BIOLOGY
THE UNIVERSITY OF CHICAGO

It has been shown in a previous paper that coupled reactions may produce diffusion phenomena which are characterized by highly asymmetric distributions of concentrations in a spherical cell. Under certain conditions such diffusion fields are unstable, the asymmetries in concentrations tending to increase indefinitely. This results in an increase of asymmetrically distributed osmotic pressures which may eventually result in a division of a cell. Constriction without elongation, as in cleavage, is studied. The process of division is brought about in this case by ordinary osmotic pressure, and not by the diffusion drag forces.

In a recent paper (Rashevsky, 1948a) we have shown that two simple coupled reactions may produce time-variable diffusion fields in a spherical cell which may exhibit a highly asymmetric spatial structure. However, those fields are in general unstable and cannot last permanently. The only stable permanent distribution of concentrations is a spherically symmetric one.

Even temporary asymmetries of concentrations, when large enough, will produce an asymmetric distribution of ordinary osmotic pressures, and this may result in forces which will tend to deform the cell and perhaps even divide it into two.

The purpose of the present paper is to discuss this possibility. The reader's familiarity with the previous paper is presupposed.

By adding instead of subtracting equations (8) and (9) of *loc. cit.*, we find that

$$\Psi_i = c_{i1} + c_{i2} \tag{1}$$

satisfies the same differential equation as Φ_i. Hence, Ψ_i will be given by similar expressions as Φ_i. If, contrary to the usual convention, we express c_{i1} and c_{i2} in mol cm^{-3} rather than in gm cm^{-3}, which does not change anything in the equations, the total osmotic pressure at each point will be proportional to Ψ_i. Hence the osmotic pressure p will be given as a function of time and coordinates by expressions similar to those which give Φ_i.

Separating p into a time-dependent part p^* and a time-independent part p_0,

$$p = p_0 + p^*, \tag{2}$$

using the same notations as in *loc. cit.*, we may write [*loc. cit.*, equation (28)]:

$$p^* = \psi e^{\nu t}, \tag{3}$$

where ν is given by [*loc. cit.*, equation (45)]:

$$\nu^2 + [\lambda^2(D_{i1} + D_{i2}) - a_{11} - a_{22}]\,\nu + \lambda^4 D_{i1}D_{i2} - \lambda^2(a_{11}D_{i2} + a_{22}D_{i1}) + a_{11}a_{22} - a_{12}a_{21} = 0. \tag{4}$$

The quantity λ is the *eigenvalue*, determined by equations (63) and (71) of *loc. cit.* For the particular case where $D_{e1} = D_{e2} = 0$ and $p_e = 0$ (corresponding to $\Phi_e = 0$ in *loc. cit.*), λ is determined by

$$\frac{d}{dr}\left[\frac{1}{\sqrt{r}} J_{m+\frac{1}{2}}(\lambda r)\right]_{r=r_0} + \frac{k_1}{\sqrt{r_0}} J_{m+\frac{1}{2}}(\lambda r_0) = 0. \tag{5}$$

Without loss of generality we shall confine ourselves in this paper to that case.

Consider the axially symmetric particular solution

$$p^* = \frac{a}{\sqrt{r}} J_{5/2}(\lambda r) P_2(\cos\theta) e^{\nu t}, \tag{6}$$

which is a particular case of equation (52) of *loc. cit.* The osmotic pressure has two maxima near the poles (Figure 1, shaded area) and

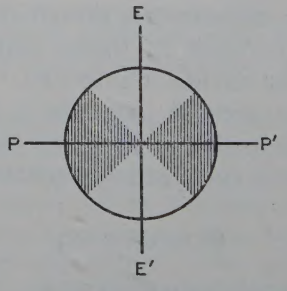

FIGURE 1

a minimum along the equator EE' (Figure 1). If for a given value of λ, say the smallest value, λ_1, the quantity ν is real and positive, then the difference in osmotic pressures between the polar and equatorial regions will increase with time. By applying Betti's equation

it can readily be shown that such a distribution of pressure as shown on Figure 1 will tend to elongate the cell along the line PP' and constrict it along the line EE'.

The first question is whether positive values of ν are possible. The affirmative answer to this is obtained by a simple example.

Denote by x_1 the smallest positive root of $J_{5/2}(x) = 0$ and by x_1' the smallest positive root of $J'_{5/2}(x) = 0$. When k_1 is very large the second term of (5) prevails, and λ_1 is given by

$$\lambda_1 r_0 = x_1, \quad \text{or} \quad \lambda_1 = \frac{x_1}{r_0}. \tag{7}$$

When k is very small or zero, the first term of (5) prevails and λ_1 is given by the equation

$$\frac{\lambda}{\sqrt{r_0}} J'_{5/2}(\lambda r_0) - \frac{1}{2\sqrt{r_0^3}} J_{5/2}(\lambda r_0) = 0. \tag{8}$$

Equation (7) gives, with $r_0 \sim 10^{-3}$ cm, $\lambda_1 \sim 10^3$. Let us *assume* for a moment that the value of λ_1, as determined by (8) is also of the order of magnitude of 10^3. The coefficients of both terms of (8) are then of the same order of magnitude (10^4). The smallest roots of both equations

$$J'_{5/2}(x) = 0 \quad \text{and} \quad J_{5/2}(x) = 0 \tag{9}$$

are of the order of magnitude of unity. Since both $J'_{5/2}$ and $J_{5/2}$ are oscillating functions, the smallest value of λr_0 for which (8) holds will also be of the order of unity.

Thus both for small and large values of k_1, the value λ_1 is of the order of 10^3.

Now let us take as plausible values (Rashevsky, 1948b) $D_{i1} = D_{i2} \sim 10^{-7}$ cm^2 sec^{-1}, $a_{11} = a_{12} = a_{21} \sim 10^{-1}$ sec^{-1}, $a_{22} = 0$. With these values and with $\lambda_1 \sim 10^3$, we find for the positive root of (4) the value $\nu \sim 10^{-1}$ sec. With such a value of ν the pressure difference between polar and equatorial regions will increase about 3 times every 10 seconds. Smaller or larger values of ν may still be obtained within the plausible range of values of the other constants.

The next question is how to calculate the effects of such a variable pressure on the cell. As the cell deforms under the action of this pressure, the solutions which were derived for a spherical cell will no longer hold. We must resort to the approximation method. Its generalization to problems of this type has been outlined recently (Rashevsky, 1948c). We may consider an oblong cell, divided into "polar" and "equatorial" regions as shown on Figure 2. To such a cell we may

apply the approximation method in the form given in the paper mentioned above (Rashevsky, 1948c). We thus will obtain the variation of concentrations and hence of pressures in both regions as functions of the length and width of the cell. Then, assuming in the usual way that the deformation is slow, we can apply Betti's equation.

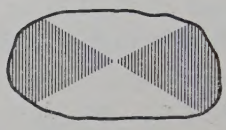

FIGURE 2

Leaving such a study for a later time we shall here consider, *as an illustration only,* the case in which the cell is prevented from elongating either by a sufficiently rigid outer membrane or by other cells in a tissue. Even in the absence of elongation there will be a tendency toward constriction in the equatorial region EE', if the osmotic pressure here inside of the cell becomes sufficiently low. Let us derive the equation of the constriction assuming, as a very crude approximation, that this constriction does not too appreciably affect the distribution of the concentration.

Such an assumption may at least partly be justified by the consideration that along the plane EE', $\partial p/\partial\theta = 0$. The gradient of the concentration is therefore zero in the direction of the normal to EE'. Hence an introduction of an impermeable partition along EE' should not affect the diffusion field. A *narrow* constriction such as shown on Figure 3 may be considered as roughly equivalent to such a partition.

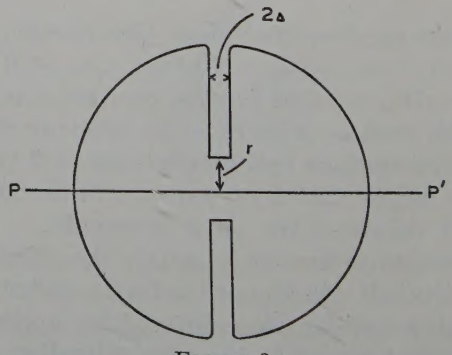

FIGURE 3

(The mechanical analogue to this is the fastening of a vibrating system at a node or along a nodal line or plane.)

Consider the "neck" (Figure 3) as a cylinder of length 2Δ and a radius r. Such a distribution of pressures inside of the cell as discussed above will result in a net pressure $-p_1$ (directed inward) as applied to the "ends" of the "neck", and a pressure $-p_2$ applied to the "sides", with p_1 and p_2 both positive and $p_1 < p_2$. Now applying Betti's equation to the neck, we find (Rashevsky, 1948b, p. 142),

$$\frac{1}{\Delta}\frac{d\Delta}{dt}=\frac{1}{3\eta V}I_2, \tag{10}$$

where V is the volume of the neck,

$$V=2\pi r^2\Delta, \tag{11}$$

and I_2 is given by†

$$I_2=V(p_2-p_1)=Vp;\quad p=p_2-p_1>0. \tag{12}$$

To the osmotic pressure we also must add the capillary pressure acting on the "sides". That pressure is composed of a negative part, $-\gamma/r$, and of a positive part γ/Δ. The latter is due to the fact that the curvature in the meridional plane of the neck is negative and its radius is of the order of magnitude of Δ. Therefore the total capillary pressure on the "sides" is $-\gamma\left(\frac{1}{r}-\frac{1}{\Delta}\right)$. This adds to I_2 a term

$$V\gamma\left(\frac{1}{r}-\frac{1}{\Delta}\right). \tag{13}$$

If the "neck" elongates at constant volume, then (Rashevsky, 1948b, p. 149)

$$\frac{1}{r}\frac{dr}{dt}=-\frac{1}{2\Delta}\frac{d\Delta}{dt}, \tag{14}$$

and therefore altogether we have, combining (10), (12), (13), and (14):

$$\frac{dr}{dt}=-\frac{1}{6\eta}\left(p-\frac{\gamma}{\Delta}\right)r-\frac{\gamma}{6\eta}. \tag{15}$$

Actually in a pure constriction such as considered here, the neck does not elongate, and the volume is not constant. Since the elongation occurs because of a net lateral pressure, we may consider the case

†Notice the difference in the expression for the volume here ($2\pi r_1 r_2^2$) and in N. Rashevsky, 1948b, p. 142 ($\frac{4}{3}\pi r_1 r_2^2$).

is equivalent to one in which a plastic rod elongates under lateral pressure, but as it elongates it is gradually chopped off at the ends so as to keep the length constant. Approximately equation (15) will still hold.

Equation (15) is readily integrated if we consider the case in which p varies very slowly so that it does not change appreciably during the process of constriction. We may then consider p as constant.

Putting

$$\frac{1}{6\eta}\left(p-\frac{\gamma}{\Delta}\right)=a;\quad \frac{\gamma}{6\eta}=b; \tag{16}$$

and integrating (15) with the initial condition

$$r=r_0 \quad \text{for} \quad t=0, \tag{17}$$

we find

$$r=\frac{ar_0+b}{a}e^{-at}-\frac{b}{a}. \tag{18}$$

Constriction occurs only if $a>0$, or $p-\gamma/\Delta>0$. The division ends when $r=0$, which happens at

$$t=\frac{1}{a}\log\frac{ar_0+b}{b}>0. \tag{19}$$

In this case the constriction follows an entirely different course than when the cell is allowed to elongate freely (Rashevsky, 1948b, p. 148 ff.). Data on this type of constriction are not available but could readily be obtained in a similar manner as those for freely elongating cells.

If $p \propto e^{\nu t}$ we obtain more complicated expressions.

If ν is real and positive only for $\lambda=\lambda_1$, and either negative or complex with a negative real part for all other λ's, then the numerous other possible configurations which correspond to the individual terms of the general expression (65) of *loc. cit.* will exist for a while, but gradually will disappear. As seen from (4), the damping is positive for sufficiently large λ's, and increases with λ. The configuration which corresponds to (6) will persist and produce a division under proper conditions. At the early stages of the whole process the terms which correspond to damped periodical oscillations will be of the same order as the term (6). Each of those terms will produce temporarily periodical deformations of the cell. Since the periods are in general incommeasurable, the net result will be a somewhat random-like pulsations of the cell. These pulsations will die out and the division begin. But this is exactly what we see on micro-motion pictures of di-

viding cells when the process is speeded up about one hundred times. (C.f. the Canti film.)

It may happen that ν will also be real and positive for a value of λ which corresponds to a solution of the form

$$\frac{a}{\sqrt{r}} J_{7/2}(\lambda r) P_3(\cos\theta) . \tag{20}$$

In this case a three-polar division will occur, because there will be three maxima of osmotic pressure.

By investigating the range of values of D_{i1}, D_{i2}, a_{11}, a_{12}, a_{21}, and a_{22} at which this can happen it may be possible to estimate the probability of occurrence of triple divisions.

Finally ν may be complex with a positive real part. In that case the difference between the polar and equatorial pressures will oscillate with increasing amplitude and the possibility of a division during the proper phase of one of the periods should be investigated.

It must be remarked that even for a real positive ν the concentrations cannot increase indefinitely, since the smallest concentration must still be positive. The maximum value they can reach is determined by the value of the time-independent solution on which the variable solutions are superimposed (*loc. cit.*). Should a division occur at all, it must occur before these limiting values are reached. This is likely to introduce some additional inequalities as a condition of division.

The important thing in the present case is that the division of a cell is caused by ordinary osmotic pressure, and not by the diffusion drag forces.

LITERATURE

Rashevsky, N. 1948a. "On Periodicities in Metabolizing Systems." *Bull. Math. Biophysics*, **10**, 159–174.

Rashevsky, N. 1948b. *Mathematical Biophysics.* Second Edition. Chicago: University of Chicago Press.

Rashevsky, N. 1948c. "A Note on Biological Periodicities." *Bull. Math. Biophysics*, **10**, 201–204.

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 11, 1949

A NOTE ON THE DIFFUSION DRAG FORCES

N. RASHEVSKY
COMMITTEE ON MATHEMATICAL BIOLOGY
THE UNIVERSITY OF CHICAGO

A formal derivation of the expression for the diffusion drag force is given. The expression involves the coefficient of diffusion of the solute through the solvent and the coefficient of diffusion which the solute would possess if it diffused through itself in the form of a gas, in the absence of the solvent.

The theory of the diffusion drag forces presents an interesting peculiarity. The existence of the forces follows directly from Newton's third law without any additional assumptions. Since the solvent offers a resistance to the flow of the solute, the latter must exert an equal and opposite force on the solvent. It would seem that under those conditions the force should be easily expressed in terms of the diffusion coefficient which is a measure of the force of resistance of the solvent. Yet apparently rather complex kinetic considerations have been hitherto needed (Rashevsky, 1948) in order to arrive only at an estimate of the order of magnitude of the forces.

The purpose of this note is to outline a simple derivation which must be expected to be possible.

Let D_0 be the self-diffusing coefficient of the solute, considered as an ideal gas, in the absence of the solvent. Then, in the absence of the solvent, a gradient of concentration c would produce a flow per cm²

$$Q = -D_0 \operatorname{grad} c . \tag{1}$$

The mass velocity, v_0, corresponding to this flow, is given by

$$Q = v_0 c , \tag{2}$$

or

$$v_0 = \frac{Q}{c} . \tag{3}$$

Equations (1) and (3) give

$$v_0 = -\frac{D_0}{c} \operatorname{grad} c. \tag{4}$$

The gradient of concentrations in a gas produces an aerodynamical force F per unit volume equal to $-\operatorname{grad} p$, where p is the pressure. Therefore

$$F = -\frac{RT}{M} \operatorname{grad} c. \tag{5}$$

In the absence of inertial forces and in a steady state, the force F is equal to a frictional force produced by the velocity v, and equal to αv_0, where α is a coefficient. Hence

$$\alpha v_0 = -\frac{RT}{M} \operatorname{grad} c, \tag{6}$$

or

$$v_0 = -\frac{RT}{\alpha M} \operatorname{grad} c. \tag{7}$$

Comparison of (4) and (7) gives

$$\frac{D_0}{c} = \frac{RT}{\alpha M}, \tag{8}$$

or

$$\alpha = \frac{RT}{M} \frac{c}{D_0}. \tag{9}$$

In the presence of the solvent, the latter exerts an additional force f_r of resistance upon the diffusion flow and makes the latter smaller than given by (1). The flow now is

$$-D \operatorname{grad} c, \tag{10}$$

where $D << D_0$.

The corresponding mass velocity is now equal to

$$v = -\frac{D}{c} \operatorname{grad} c; \qquad |v| << |v_0|. \tag{11}$$

We may regard the effect of the solvent as equivalent to a force f_r, directed opposite to F, in other words, a force of resistance added to αv. Then for a steady state we have

$$F - f_r = \alpha v. \tag{12}$$

Introducing (5), (9), and (11) into (12) we find

$$-\frac{RT}{M}\operatorname{grad} c - f_r = -\frac{RT}{M}\frac{c}{D_0}\frac{D}{c}\operatorname{grad} c, \tag{13}$$

or

$$f_r = \frac{RT}{M}\left(\frac{D}{D_0} - 1\right)\operatorname{grad} c. \tag{14}$$

The diffusion drag force is equal to $-f_r$ and hence is given by

$$f = \frac{RT}{M}\left(1 - \frac{D}{D_0}\right)\operatorname{grad} c. \tag{15}$$

The quantity $\frac{D}{D_0}$ is about $10^{-4} - 10^{-5}$. Hence almost all of the osmotic force $-\frac{RT}{M}\operatorname{grad} c$ is sustained by the solvent. Inside of the cell we have D_i; outside of the cell, $D_e > D_i$. If $D_i = D_e$, then the potential of the force f,

$$\phi = \frac{RT}{M}\left(1 - \frac{D}{D_0}\right)c, \tag{16}$$

would be continuous throughout the cell and the whole space if the permeability h is infinite and c is continuous at the surface. Application of Betti's equation to the inside and the outside of the cell would give zero deformation for this case. If, however, $D_i \leqslant D_e$, then

$$\phi_i = \frac{RT}{M}\left(1 - \frac{D_i}{D_0}\right)c \tag{17}$$

and

$$\phi_e = \frac{RT}{M}\left(1 - \frac{D_e}{D_0}\right)c, \tag{18}$$

and at the surface there is a discontinuity

$$\phi_i - \phi_e = \frac{RT}{M}\frac{D_e - D_i}{D_0}c, \tag{19}$$

which permits a resultant elongation.

Compared to previously derived expressions (Rashevsky, 1948) the value of the effective potential, and therefore of the effective force, is reduced by the factor $(D_e - D_i)/D_0 \sim 10^{-4}$. H. D. Landahl's (1942) work indicates, however, that in *Arbacia* eggs the forces needed to

produce division are about $10^{-4} - 10^{-5}$ times smaller than those computed from plausible values of the effective metabolism and from the assumptions that the force per unit volume is of the order of $-(RT/M)$ grad c.

Inside of the cell the potential of the diffusion force is

$$\phi_i = \frac{RT}{M}\left(1 - \frac{D_i}{D_0}\right) c; \tag{20}$$

outside of the cell it is equal to

$$\phi_e = \frac{RT}{M}\left(1 - \frac{D_e}{D_0}\right) c. \tag{21}$$

Let $h = \infty$, so that c is *continuous* at the surface. Does the discontinuity

$$\phi_i - \phi_e = \frac{D_e - D_i}{D_0} c \tag{22}$$

of the potential introduce any additional surface forces?

To answer this question let us consider the realistic case in which the boundary between two phases is not a mathematical surface but a transitory layer of the thickness δ in which all properties vary continuously from one phase to the other.

The potential ϕ will also vary continuously from ϕ_i to ϕ_e. The force per unit area is equal to

$$p = -\int_0^\delta \left[1 - \frac{D(x)}{D_0}\right] \frac{dc}{dx}\, dx = \tag{23}$$

$$-\int_0^\delta \frac{dc}{dx}\, dx + \frac{1}{D_0}\int_0^\delta D(x)\, \frac{dc}{dx}\, dx,$$

if x is chosen in the direction of the normal.

The first term on the right-hand side is equal to $-[c(\delta) - c(0)] = 0$, because of the continuity of c. In the second term $D(dc/dx) = \text{const.} = Q$. Hence the second term equals $Q\delta/D_0$ and tends to zero with δ. A discontinuity of D thus does not introduce any additional surface forces.

It should be noted that expression (15) represents the force on the unit volume of the solvent *as a whole*. Any suspended particles are to be considered parts of the solvent. The addition of such particles affects D, and thus affects the force. Herein is an essential dif-

ference between the derivation suggested here and previous ones in which the solvent had to be broken somewhat artificially into solvent proper and suspended particles (Rashevsky, 1948).

LITERATURE

Landahl, H. D. 1942. "A Mathematical Analysis of Elongation and Constriction in Cell Division." *Bull. Math. Biophysics*, 4, 45–62.

Rashevsky, N. 1948. Mathematical Biophysics. Second Edition. Chicago: University of Chicago Press.

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 11, 1949

NOTE ON A CASE OF NONLINEAR DIFFUSION

N. RASHEVSKY
COMMITTEE ON MATHEMATICAL BIOLOGY
THE UNIVERSITY OF CHICAGO

When the function Q in the equation $\nabla^2 c + Q(c) = 0$ is positive and is of a specified kind, the equation admits of a centrally spherical solution such that c is positive everywhere, tending to zero at infinity and $dc/dr = 0$ at $r = 0$. Physically this corresponds to a local concentration of the solute in an infinite medium without any membranes present. This result would indicate the possibility of the formation of spontaneous concentrations and non-uniformities in non-linear diffusion fields. Possible biological implications are mentioned.

Consider the general diffusion equation

$$D\nabla^2 c + Q\,, \tag{1}$$

where D is the diffusion coefficient, c the concentration, and Q a function of c with the following properties:

1) $Q(c)$ is continuous and positive for $c > 0$.
2) $Q(0) = 0$.
3) $Q(\infty) = Q_0$ (= const).
4) In the interval 0, c_1, where c_1 is a constant, $Q(c)$ varies as ac^n, where $n > 3$, and a is a coefficient.
5) In a small interval $c_1 - c_2$, where c_2 is another constant such that $c_2 - c_1 << c_1$, $Q(c)$ raises very suddenly, then again begins to vary slowly, and for $c > c_2$ tends asymptotically to Q_0 so that $Q_0 - c << Q_0$ for $c > c_2$.
6) The coefficient a is sufficiently small so that $ac_1^n << Q_0$.

Putting $D = 1$ in equation (1) without loss of generality and writing it in polar coordinates for the case of spherical symmetry, we obtain

$$\frac{d^2c}{dr^2} = -\frac{2}{r}\frac{dc}{dr} - Q(c)\,. \tag{2}$$

Let us construct a solution of (2) such that when $r = 0$, then

$$c = c_0 > c_2 \text{ and } dc/dr = 0\,. \tag{3}$$

Since for $c > c_2$, $Q(c)$ is very nearly constant and equal to Q_0, the condition (3) shows that at $r = 0$, $d^2c/dr^2 < 0$. If $Q(c)$ were exactly constant and equal to Q_0, then we would have $c = c_0 - Q_0 r^2/6$; $dc/dr = -Q_0 r/3$; $d^2c/dr^2 = -Q_0/3$. As $Q(c)$ actually slightly deviates from a constant, the actual values of dc/dr and d^2c/dr^2 will be slightly different, but d^2c/dr^2 will remain negative for $r = 0$.

But if at $r = 0$, $d^2c/dr^2 < 0$, then, as r increases, both d^2c/dr^2 and dc/dr remain negative, and therefore c decreases. As long as c remains above c_2, $Q(c)$ remains appreciably constant, while the absolute value of $(1/r)\ (dc/dr)$ increases. When however c drops below c_2, then any further small decrement of c causes $Q(c)$ to drop sharply according to 5) and 6). Now the term $Q(c)$ in (2) becomes negligible as compared with dc/dr, and c varies according to the equation

$$\frac{d^2c}{dr^2} + \frac{2}{r}\frac{dc}{dr} = 0, \tag{4}$$

which, integrated, gives

$$c \propto \frac{1}{r}. \tag{5}$$

Now we have $(1/r)\,(dc/dr) \propto 1/r^3$, and therefore because of 4), $Q(c)$ will remain negligible as compared to $(1/r)\,(dc/dr)$, if it has become negligible at any one point.

Our solution represents a more or less steep concentration of a solute in an indefinitely extended medium. The function $Q(c)$ corresponds to a production of the solute. Since equation (2) is an equation for a stationary state, we see that for a function $Q(c)$ as specified above, such concentrations of the solute may exist in a stationary state.

We shall not investigate here whether the stationary state described in this paper is stable. We shall, however, point out that with *linear* diffusion we cannot obtain such solutions, stable or not. For $Q = q$ (= const) the only solution which is finite at $r = 0$ is

$$c = c_0 - \frac{qr^2}{6}. \tag{6}$$

This does not vanish at infinity, becomes negative for $r > \sqrt{6c_0/q}$ and thus can have no physical meaning in the whole space. For $Q(c) = k^2c$ we have

$$c = A\frac{\sin kr}{r}. \tag{7}$$

This vanishes at infinity but in an infinite number of regions is negative and therefore again physically meaningless. Only within limited space, bounded by proper membranes, can solutions (6) and (7) acquire physical meaning. The solution of the nonlinear equation (2) is physically meaningful in the whole space and represents a localized accumulation of the dissolved substance, which does not need any membranes for its maintenance.

For the case of spherical symmetry, when the concentration depends only on one coordinate, r, we can always find a form of $Q(c)$ in (2) such that the solution of (2) will be a prescribed function of r. For if c is given, so that

$$c = f(r), \tag{8}$$

then $\nabla^2 c$ is also given as a function of r,

$$\nabla^2 c = u(r). \tag{9}$$

Eliminating r from (8) and (9) we find $\nabla^2 c = v(c)$, and we put $Q(c) = v(c)$.

Should the possibility of such accumulations occur in other nonlinear cases, and should those accumulations be stable, this would offer interesting possibilities of spontaneous local accumulations of dissolved substances in a cell. Those may result in interesting permanent structures.

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 11, 1949

ON THE VELOCITY OF CONDUCTION IN NERVE FIBERS WITH SALTATORY TRANSMISSION

H. D. LANDAHL AND R. J. PODOLSKY†
COMMITTEE ON MATHEMATICAL BIOLOGY
THE UNIVERSITY OF CHICAGO

Using an electrical model to represent certain features of a nerve fiber together with a one-factor theory of excitation, an expression is obtained for the velocity of propagation of a nerve impulse along a nerve fiber exhibiting saltatory transmission. The velocity shows a maximum with respect to internodal length. A critical internodal length, a critical radius, and a critical value for the resistance of the external medium are required in order to make transmission of the impulse possible.

Expressions for the velocity of conduction of an impulse in nerve fibers have been obtained using various models (Rashevsky, 1933, 1938; Rushton, 1937; Offner, Weinberg, and Young, 1940). N. Rashevsky (1938, 1948) considered the case of fibers with nodes of Ranvier. However the explicit value of the currents was not given in terms of the physical constants. It is the purpose of the present paper to introduce a specific network and thus find the current distribution in terms of the physical constants of the nerve; then to discuss the dependence of the conduction velocity on the physical constants.

Let a myelinated nerve fiber, whose radius, exclusive of the sheath, is r cm, have nodes of effective electrical width δ spaced a distance a cm apart. Let γ be the ratio of the internal resistance per unit length to the resistance per unit length of the external medium. Let ρ be the specific resistance of the internal medium and let Ω be the transverse resistance in ohms cm^2 of one square centimeter of membrane plus any sheaths at the node. We shall assume that the myelin sheath of the internode is a perfect insulator. Let the normal resting potential of the nerve fiber be E volts.

In order to solve our problem it is necessary to ascertain the current distribution along the nerve. To accomplish this most easily we represent the principal properties of the nerve by the electrical network shown in Figure 1.

†Fellow, National Institute of Health.

Whenever the node is short compared to the internode, the currents will probably be uniformly distributed across the cross section of the nerve fiber and transversely along the node. In this case, if we ignore the effects of capacity or inductance, the geometry of the nerve leads us to the network of Figure 1, except for the element representing the zero node which will be discussed below.

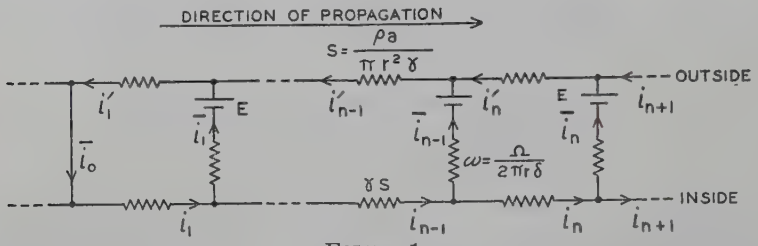

FIGURE 1

In treating the problem of conduction we shall follow N. Rashevsky (1938, chap. xx; 1948, chap. xxviii) in which the law of excitation is given by a one-factor theory (Blair, 1932; Rashevsky, 1938, chap. xvi; 1948, chap. xxiii). According to Blair's theory, excitation in the nerve is governed by a change from the normal in the amount of a factor in the neighborhood of a nerve. The rate of accumulation of this factor increases in proportion to the current applied to the nerve and decreases by an amount equal to a constant k times the change in concentration of the exciting factor. The constant k tells one how rapidly the factor dissipates when the current is removed. It is postulated that excitation occurs when the amount of the factor reaches a threshold. The smallest current which can produce excitation is the rheobase R. We shall consider the transverse current through a node of Ranvier to be the current causing the accumulation of the factor determining excitation, so that this is the current to be used in the equations of excitation. The current density rheobase in amperes per square centimeter will be denoted by $R = R/2\pi r\delta$. We shall assume that the transverse resistance ω drops to a small enough value at the point of excitation so that the current flow can be calculated by assuming its value to be zero. The potential at the point of excitation is taken to be zero. This accounts for the element representing the zero node in Figure 1.

The assumption that the transverse resistance becomes zero at the point of excitation is introduced for simplicity. The properties we shall discuss in the present paper do not depend significantly upon the value to which the transverse resistance at the excited node is reduced. A direct consequence of this simplification in the model is

that there is no non-propagated response possible. Thus for the present we shall not distinguish between the constant k' for the excitatory process at a point and the constant k associated with the initiation of a propagated impulse by means of an external stimulation.

Let an impulse travel from left to right. The node at which excitation has just occurred is taken to be the zero node. Since the resistance of this element is taken to be zero, the current distribution along the nerve to the right of this node is independent of the currents on the left. Due to the asymmetry of the network, there will be a current distribution along the nerve to the right. Of the currents through the nodes, that in the first is the greatest. Thus the excitatory factor in that node will be the first to reach the threshold required for excitation. In the network, excitation is represented by E and ω going to zero. Then the current distribution shifts one node to the right. The rate of this shift is the conduction velocity.

We shall now compute the current distribution to the right along the nerve fiber. The net flow of current down the nerve must be zero; thus the current inside of an internode is equal and opposite to that outside (Rashevsky, 1938, chap. xix; 1948, chap. xxvii) or, from Figure 1, $i_n = i_{n'}$. From Kirchoff's laws we get the equations from which to obtain a single equation determining the current distribution (c.f. Rashevsky, 1938; 1948). The junction equations give

$$i_n = \bar{\imath}_n + i_{n+1} = i_{n-1} - \bar{\imath}_{n-1}\,, \tag{1}$$

and the equation for the potentials may be written

$$0 = i_n(\gamma S + S) + \bar{\imath}_n\omega - \bar{\imath}_{n-1}\omega\,. \tag{2}$$

Eliminating the transverse currents we obtain the difference equation

$$i_{n+1} - \frac{(\gamma+1)S + 2\omega}{\omega}\, i_n + i_{n-1} = 0\,, \tag{3}$$

for which the solution is

$$i_n = A\,(1 + \psi - \sqrt{2\psi + \psi^2})^n, \tag{4}$$

where A is a constant determined by the boundary conditions and where

$$\psi = \frac{(1+\gamma)a\rho\delta}{r\Omega\gamma} = \frac{(1+\gamma)S}{2\omega}. \tag{5}$$

In order to evaluate the constant A we consider the currents at the excited region. We find, corresponding to equations (1) and (2), the equations

$$i_1 = \bar{i}_1 + i_2; \tag{6}$$

$$E = \gamma S i_1 + S i_1 + \bar{i}_1 \omega . \tag{7}$$

Eliminating $\bar{i}_1$, i_1, and i_2 from equation (7) by equations (4) and (6), we obtain an equation which, solved for A, gives

$$A = E/\omega[1 + \psi - \sqrt{2\psi + \psi^2}]\,[\psi + \sqrt{2\psi + \psi^2}] . \tag{8}$$

From equation (1), $\bar{i}_1 = i_1 - i_2$. Introducing (8) into (4) we can obtain i_1 and i_2. In this way we find

$$\bar{i}_1 = E(\sqrt{2\psi + \psi^2} - \psi)/\omega(\sqrt{2\psi + \psi^2} + \psi). \tag{9}$$

Using essentially the same argument as was used by N. Rashevsky (1938, chap. xx; 1948, chap. xxviii) we obtain an expression for the time required for the excitation to move from one node to the next:

$$\Delta t = \frac{1}{k} \log_e \left(\frac{\bar{i} - 2\pi r \delta (1 + \psi - \sqrt{2\psi + \psi^2}) \bar{R}}{\bar{i} - 2\pi r \delta \bar{R}} \right) . \tag{10}$$

Hence the velocity is $a/\Delta t$.

We define λ as

$$\lambda = \frac{E}{\Omega \bar{R}} - 1. \tag{11}$$

The quantity λ is customarily referred to as the "safety factor" and is a measure of the ratio of the current that flows through the nodes of the functioning nerve to the minimum current that can cause excitation. Upon elimination of $\bar{i}_1$ from (10), using (9), and making appropriate re-arrangements we obtain the final result:

$$v = ak/\log_e \left(1 + \frac{2}{\lambda\sqrt{1 + 2r\Omega\gamma/a\rho\delta(1 + \gamma)} - \lambda - 2}\right) . \tag{12}$$

Discussion.

We now consider the effects of the various parameters on the velocity of conduction. The velocity is directly proportional to k. The distance between nodes, a, enters in two places, one tending to increase the velocity and the other tending to decrease the velocity. An increase in λ causes an increase in the velocity. The parameters E, Ω, and $\bar{R}$ determine λ. The other factors enter as a combined quantity which, however, also contains Ω.

The accompanying figures show the dependence of v on several of the physicochemical parameters of the nerve fiber and its environment. Unless otherwise indicated, the parameters used in making the

plots are as follows: $k = 2000$ sec^{-1}, $a = 0.1$ cm, $r = 5 \times 10^{-4}$ cm, $\Omega = 10^4$ ohm cm^2, $\gamma = 1$, $\rho = 10^2$ ohm cm, $\delta = 2 \times 10^{-3}$ cm. From these values we may calculate ω and S from $\omega = \Omega/2\pi r\delta$ and $S = \rho a/\gamma\pi r^2$. If we know the chronaxie time τ for a given nerve fiber, the constant k is obtained from the relation $k\tau = \log_e 2$ (Rashevsky, 1938, chap. xvi). The value chosen is representative of those determined by various authors. To find a value for Ω we assumed that the myelin sheath is superimposed over a membrane whose resistance for a square centimeter is of the same order as that of the crab nerve (Hodgkin, 1947). The impedance of the sheath is taken to be essentially infinite (Tasaki, 1939). Using this value of Ω and setting $E = 0.05$ volts, then for $\lambda = 2$, $\bar{R} = 1.7 \times 10^{-6}$ amperes/cm^2 or 1×10^{-11} amperes per node; using only the electrical parameters of the nerve, we find, for comparison, $\bar{i}_0 = 2.3 \times 10^{-10}$ and $\bar{i}_1 = 3 \times 10^{-11}$ amperes.

If we consider the limiting value of v when $a \to \delta$ and $\delta \to 0$ we find an expression for the velocity of the non-medulated nerve fiber which we may write in the form

$$v = k\lambda \sqrt{\frac{r\Omega\gamma}{2\rho(1+\gamma)}}. \tag{13}$$

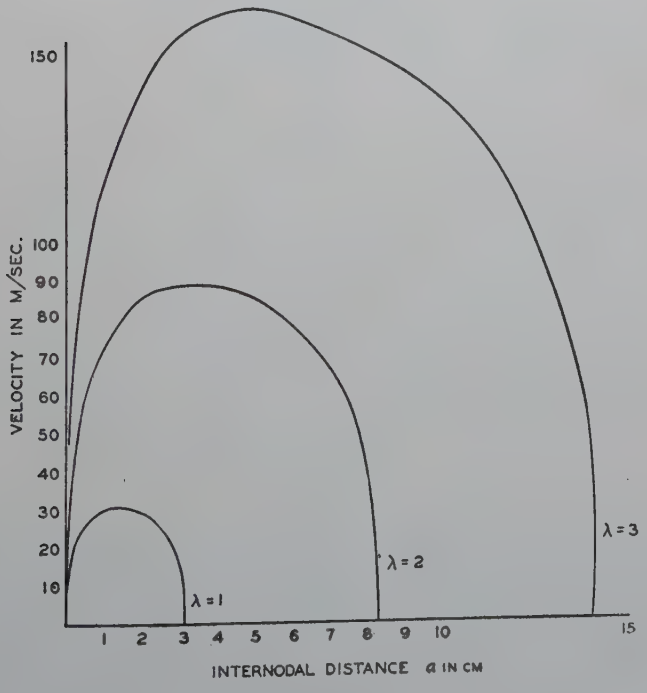

FIGURE 2

This expression would hold for a continuously medulated nerve fiber if Ω were taken to be the transverse impedance of one square centimeter of the membrane together with the myelin sheath. Equation (13) is the same expression, except for differences in notation, as that obtained by N. Rashevsky for a nerve with a continuous sheath (Rashevsky, 1938, chap. xix; 1948, chap. xxvii). The same relation was also obtained for a continuously medulated fiber considered as a special case of a fiber with an interrupted myelin sheath (Rashevsky, 1938, chap. xx; 1948, chap. xxviii). The above expression is also formally the same as that given by W. Rushton (1937, cf. Offner, Weinberg, and Young, 1940).

If we obtain v for $a \to \delta$, the value is somewhat smaller ($v = 4.4$ m/sec instead of 4.5 m/sec) than the value for $\delta = 0$. The difference arises because of the implied interference with the current flow of a zero length internode.

In Figure 2 we consider the variation of v with the internodal distance, a, for three values of the safety factor λ. It is seen that at $a = \delta$, v has a value determined by λ; it increases to a maximum as a increases, and finally falls to 0 for a critical value of a. It it were possible to effectively insulate a node, such behavior could be investigated experimentally. Since the critical value of a depends upon λ, this technique might be used to evaluate λ for various nerve fibers of the myelinated type, or at least impose a restriction on its value.

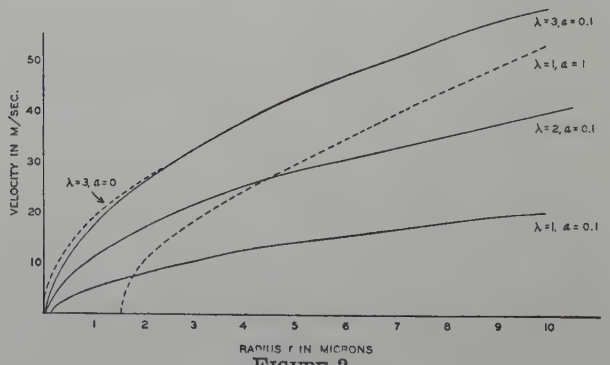

FIGURE 3

In Figure 3 we plot the value of v as a function of the nerve radius. The radius considered should be the radius of the cylinder occupied by the axoplasm. In general the velocity increases with r. This fact has been reported by many investigators (Erlanger and Gasser, 1937). However it is seen that a critical radius exists, i.e., for a certain set of physical parameters, the fiber must be greater

than a determined radius for propagation to occur. For $a = 0.1$ cm, this critical radius is seen to be 0.05μ for $\lambda = 2$ and 0.16μ for $\lambda = 1$. For $a = 1$ cm, $\lambda = 1$ the critical radius is 1.5μ.

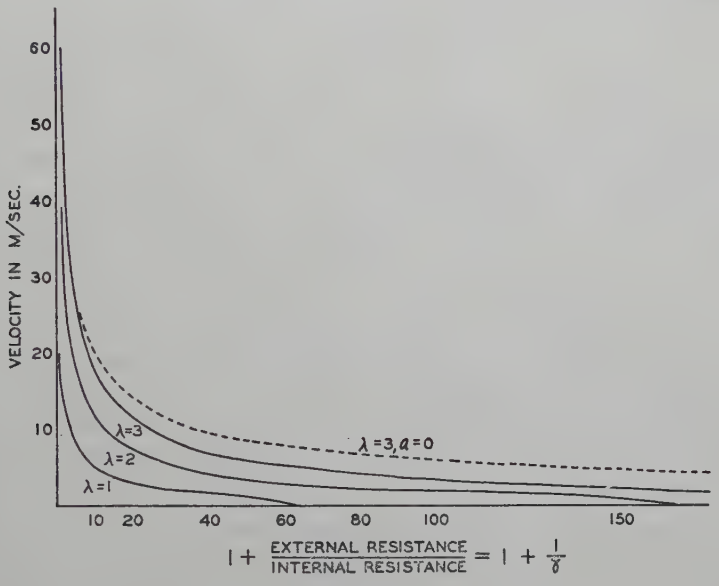

FIGURE 4

In Figure 4 we consider the effect on the conduction velocity of the electrical impedance of the medium in which the nerve is immersed. This is a factor which may be varied by placing the nerve in an oil medium, by adding a non-electrolyte such as sucrose to the external medium, or by imposing physical constraint on the flow of the external current. It is seen that as γ decreases, that is, as the external resistance is increased, we have a smaller conduction velocity. The interesting feature of this curve is that for the non-myelinated fibers there is no value of γ for which $v = 0$. However, if the nerve is myelinated the theory predicts that there is a value of γ for which nerve conduction is not possible. For example, if $\lambda = 1$, the nerve should cease conducting if placed in a medium such that $\gamma > \frac{1}{60}$. However, if the safety factor is greater, we see that the action of the myelinated nerve approaches that of the non-myelinated fiber, and the different behaviors may be experimentally indistinguishable.

The form of the curves suggests the following experimental technique for the evaluation of λ and one of the factors Ω, ρ, or δ. If $\lambda > 4/(\sqrt{1 + 2/\psi} - 1)$ (a condition which is satisfied for the typical

nerve in a normal environment), we may simplify the expression for v by expanding the logarithm. It is readily seen that the following relation is obtained:

$$2\left(\frac{v}{ak}+1\right)=\lambda\left(\sqrt{\frac{2r\Omega}{a\rho\delta}}\sqrt{\frac{\gamma}{1+\gamma}}-1\right). \tag{14}$$

Therefore if we know the value of k from consideration of the chronaxie time, and if we measure the value of a by direct observation, we can find λ and $\sqrt{2r\Omega/a\rho\delta}$ by plotting the quantity $2(v/ak + 1)$ against $\sqrt{\gamma/(\gamma + 1)}$. It must be remembered that the only values of γ allowable are those which satisfy the inequality above. The data should lie on a straight line whose slope is $\lambda\sqrt{2r\Omega/a\rho\delta}$ and whose intercept on the $\sqrt{\gamma/(\gamma + 1)}$ axis is $\sqrt{2r\Omega/a\rho\delta}$. The intercept, then, allows one to find the quantity $\Omega/\rho\delta$ using the measured values of r and a (provided one is able to make a satisfactory extrapolation to the axis of the experimental points). If ρ and δ are estimated independently, then Ω can be calculated. Since $\lambda = E/\bar{R}\Omega - 1$, we can now find the value of $\bar{R}$ from a measurement of the potential E of the nerve fiber, which in this model is the resting potential.

In all of the above graphs the various parameters were chosen as representative values. If in a particular case they are independently measured then the velocity can be computed and compared with the observed value. If now, for example, γ is varied, but any changes in the other parameters are measured, then the velocity can again be computed. At the value at which conduction is just blocked, for example, substitution of the new values of the parameters must satisfy the relation

$$2a\rho\delta(1+\gamma)(1+\lambda)=\lambda^2 r\Omega\gamma. \tag{15}$$

Actually the left-hand and right-hand members need only be very roughly equal.

Summary:

1. A model for a conducting nerve is introduced. From this model the velocity of a nerve impulse may be deduced as a function of the physical constants of the nerve fiber and its environment. This expression is derived.

2. If we consider the internodal distance of a myelinated nerve as the only parameter of the system, it is found that there is a maximum velocity and that there is a critical distance between nodes. For

internodal distances greater than this critical distance, there can be no propagation.

3. Similarly, there is a critical radius for a myelinated nerve fiber. Considering only the variation of the radius, it is found that there exists a radius which must be exceeded by a fiber found in nature if it is to be functional.

4. If we consider a single myelinated nerve fiber placed in a medium whose resistance can be varied in such a way that the properties of the nerve fiber are not changed, there will be an impedance for the external medium which can prevent the nerve from conducting impulses.

LITERATURE

Blair, H. A. 1932. "On the Intensity Time Relations for Stimulation by Electric Currents: I." *Jour. Gen. Physiol.*, **15**, 709.

Erlanger, J. and Gasser, H. S. 1937. *Electrical Signs of Nervous Activity.* Philadelphia: The University of Pennsylvania Press.

Hodgkin, A. L. 1947. "The Membrane Resistance of Non-Medulated Nerve Fibers." *Jour. Physiol.*, **106**, 305–318.

Offner, F., Weinberg, A., and Young, G. 1940. "Nerve Conduction Theory." *Bull. Math. Biophysics*, **2**, 89–103.

Rashevsky, N. 1933. "Outline of Physico-Mathematical Theory of Excitation and Inhibition." *Protoplasma*, **20**, 42–56.

Rashevsky, N. 1938. *Mathematical Biophysics.* Chicago: University of Chicago Press.

Rashevsky, N. 1948. *Mathematical Biophysics.* Second Edition. Chicago: University of Chicago Press.

Rushton, W. 1937. "Initiation of the Propagated Disturbance." *Proc. Roy. Soc. London B.*, **124**, 210–243.

Tasaki, I. 1939. "Electrical Stimulation and the Excitatory Process in the Nerve Fiber." *Am. Jour. Physiol.*, **125**, 380–395.

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 11, 1949

THE STEADY STATE KINETICS OF SOME BIOLOGICAL SYSTEMS: I

JOHN Z. HEARON†
COMMITTEE ON MATHEMATICAL BIOLOGY
THE UNIVERSITY OF CHICAGO

The steady state kinetics of some typical catalytic systems of biological importance have been formulated. The conditions for the existence of a maximum or limiting velocity are examined and discussed. In particular it is shown that the limiting velocity for a given component is simply the rate expression for a given number of steps of the *overall* process; from the general condition for a limiting velocity these steps may be specified. The stringency of the conditions which must be imposed upon the steady state solution in order that it may be assumed that one or more steps are essentially at equilibrium is pointed out. The application of the general method to coupled or branched systems and to cyclic systems is briefly discussed.

Many biological processes consist of consecutive reactions. Whether the process in question is a single enzyme system which catalyzes the oxidation of a substrate through a so-called "hydrogen transport chain," or the removal of the products of reaction from a complex system by another system or subsequent sets of systems, this feature of consecutive rate processes makes its appearance. Most frequently such reaction nets will be found in the steady state because various intermediates reach a stationary or time independent level and do not accumulate indefinitely. In general this time independent state will be different, and often far removed, from the equilibrium state. In extreme instances the conditions which specify the steady state may approximate the equilibrium condition for *some* of the steps in the overall process. We exclude here the case in which all steps are in equilibrium which would require that no net change occurs in the system. The prominence of consecutive processes in biological systems, along with the fact that the solution of the generalized set of consecutive reactions is not available unless the conditions of time independence may be assumed, makes the analysis of steady state kinetics a coordinate feature of many biological problems. Furthermore, in view of the fact that in general it is not valid to assume *a priori* that any

†This work was done while the author was a Senior Research Fellow of the National Institute of Health.

of the steps in the process are near equilibrium, it is of importance to initially examine true steady state solutions.

The classical Michaelis-Menton formulation (1913) of enzyme catalysis rests upon an assumption which, as will be pointed out, may seldom be valid. When this assumption is removed, the formulation applies at best to a simple two-step process although it has been widely utilized analogically for the description of multi-step systems. The "Michaelis-Menton constant," K_m, a perfectly definite concept in the simple theory, has often been taken to have the same physical significance in multi-step systems. The kinetic description of complex systems is *frequently* formally identical to that of the simple systems because there is a limiting velocity and an "apparent K_m" which characterizes a hyperbolic relation between rate and substrate concentration. It is clearly desirable to examine the make-up of the constant, which makes its appearance as the counterpart of K_m in multi-step systems, and to inquire into the conditions for a limiting velocity. Where it is feasible, the stringency of the conditions which must be imposed upon the steady state solutions in order that the assumption of equilibrium in some of the steps may be justified, will be examined.

The General Formulation.

The formulation which at once recommends itself is that given by J. H. Christiansen (1935) and generalized by L. P. Hammett (1937; see also references in Hammett). This formulation has been used by M. F. Morales (1947) in discussing limiting reactions and has been applied to the blood coagulation system (Hearon, 1948). It is still remarkable that this method of choice was not applied earlier and more extensively in biology. For definiteness and as a basis for reference in what follows and in forthcoming papers, some details of the method will be given.

For the general set of reactions

$$
\begin{aligned}
C_0 + C_1 &\rightleftarrows X_1 + B_1 ,\\
C_2 + X_1 &\rightleftarrows X_2 + B_2 ,\\
&\cdots\\
C_i + X_{i-1} &\rightleftarrows X_2 + B_i ,\\
&\cdots\\
C_n + X_{n-1} &\rightleftarrows B_n \quad ,
\end{aligned}
$$

denote by k_i and k_{-i} the forward and reverse rate constants for the ith step and let $K_i = \dfrac{k_i}{k_{-i}}$. If by definition

$$
\begin{aligned}
w_1 &= k_1\ [C_0]\ [C_1] ,\\
w_i &= k_i\ [C_i] ; \quad i \neq 1 ,\\
w_{-i} &= k_{-i}\ [B_i] ; \quad i \neq n ,\\
w_{-n} &= k_{-n}\ [B_n] ,
\end{aligned}
\tag{1}
$$

then the steady state conditions, $\dfrac{d\,[X_i] = 0}{dt}$, lead to

$$
\begin{aligned}
1 &= w_1\alpha_0 \quad - w_{-1}\alpha_1 ,\\
1 &= w_2\alpha_1 \quad - w_{-2}\alpha_2 ,\\
&\cdots\\
1 &= w_i\alpha_{i-1} - w_{-i}\alpha_i ,\\
&\cdots\\
1 &= w_n\alpha_{n-1} - w_{-n}\alpha_0 ,
\end{aligned}
\tag{2}
$$

where $\alpha_0 = \dfrac{1}{v}$, $\alpha_i = \dfrac{[X_i]}{v}$, and v is the overall rate of conversion of

C_0 and C_1 to B_n. It must here be assumed that the concentrations of the reactants C_i and products B_i are maintained constant. This condition is usually met by having the concentrations of these constituents sufficiently large so that their change during the time of observation is inappreciable, or in other ways as has been discussed by A. C. Burton (1939). There is still another way particularly pertinent to biological problems in which this condition of constancy may be satisfied: If a reactant in one reaction is regenerated in a subsequent reaction, the substance is conserved to the system and its concentration maintained stationary. If the last step is irreversible, this restriction of constancy may obviously be removed from B_n. The solution of equation (2) is

$$v = \frac{\prod_i^n w_i - \prod_i^n w_{-i}}{w_2 w_3 \cdots w_n + w_{-1} w_3 \cdots w_n + \cdots + w_{-1} w_{-2} \cdots w_{-(n-1)}}. \qquad (3)$$

From equation (3) it is seen that if any $w_{-i} = 0$ the second term in the numerator of v vanishes. In what follows, we shall often assume that $w_{-n} = k_{-n} \equiv 0$. The extension of the solution (3) to biological problems involves a feature of difficulty not always encountered in chemical kinetics; viz., the application of the material balance to (catalytic) components which are conserved in the system. If C_i is such a component, then

$$[C_i]_0 = [C_i] + \sum_k [X_k^{C_i}], \qquad (4)$$

where $[C_i]_0$ is the total concentration of C_i, and $X_k^{C_i}$ is any intermediate which contains C_i in bound form. Condition (4) may be imposed on the problem by including this equation in the set (2). In *particular* cases this is the simpler procedure. Alternatively, equation (2) may be solved for $[X_k^{C_i}]$; this expression, which in general contains v, substituted in equation (4) gives $[C_i]$ as a function of the w's and $[C_i]_0$. The solution of equation (2) for any $[X_i]$, first given in equivalent form by M. F. Morales (1947), may be written

$$[X_i] = \prod_k^i \frac{w_k}{w_{-k}} \left\{ 1 - v \left[\frac{1}{w_1} + \frac{w_{-1}}{w_1 w_2} + \cdots \frac{w_{-1} \cdots w_{-(i-1)}}{w_1 w_2 \cdots w_i} \right] \right\} \qquad (5)$$

If the ith step is separated from the 1st step by a number of steps, equation (5) and its use in (4) are not simple; if the ith step is sepa-

rated from the nth step by a smaller number of steps, a shorter form is available. This expression, which will not be derived here, is

$$[X_i] = \prod_{k=n}^{i+1} \frac{w_{-k}}{w_k} \left\{ 1 + v \left[\frac{1}{w_{-n}} + \frac{w_n}{w_{-(n-1)} w_{-n}} + \cdots \right. \right.$$
$$\left. \left. + \frac{w_{(i+2)} \cdots w_n}{w_{-(i+1)} \cdots w_{-n}} \right] \right\} . \tag{6}$$

Equation (6) is the result obtained from equation (2) by determinants, or by solving the nth member of (2) for α_{n-1}, substituting this into the $(n-1)$st member, etc. It should be noted that the quantities in brackets of equations (5) and (6) are such that they may be written at once from comparison to (3). In equation (5) the expression in brackets is simply $\frac{1}{V_{1,i}}$, where $V_{1,i}$ is the rate of steps 1 through i terminating irreversibly in i. Similarly, the expression in brackets of equation (6) is $\frac{1}{V_{-n_1 (i+1)}}$, where $V_{-n_1 (i+1)}$ is the *reverse* rate of steps n through $i+1$ terminating irreversibly in $-(i+1)$. On this basis, $V_{1,i}$ and $V_{-n_1 (i+1)}$ may be readily constructed, by using equation (3), in more convenient form. In the event that $k_{-n} = 0$, equation (6) becomes

$$[X_i] = v \left[\frac{w_{i+2} \cdots w_n + w_{-(i+1)} w_{i+3} \cdots w_n + \cdots w_{-(i+2)} \cdots w_{-n(n-1)}}{w_{i+1} \cdots w_n} \right] \tag{7}$$
$$= v / G_{i+1,n} ,$$

where $G_{i+1,n}$ is the function such that $[X_i]\ G_{i+1,n} = V_{i+1,n}$ and is obtainable from equation (3) by setting up $V_{i+1.n}$ and deleting $[X_i]$. These properties will be convenient in discussing the change in $[X_i]$ introduced by changing parameters of the system. In general equation (5) will give i terms in the bracket; equation (6) will give $n-i$ terms. The choice is obvious in a specific case.

The Simple Enzyme System.

It will be convenient as a basis of comparison to solve the simple system

$$E + S \rightleftarrows E \cdot S;$$
$$E \cdot S \rightarrow E + p;$$

where E, S, and p denote enzyme, substrate, and product respectively. The solution is

$$v = \frac{w_1 w_2}{w_{-1} + w_2} = \frac{k_1 k_2 [E]\ [S]}{k_{-1} + k_2}; \tag{8}$$

using equations (7) and (4) $[E_0] = [E] + [E \cdot S]$

$$[E \cdot S] = \frac{v}{w_2}; \tag{9}$$

$$v = \frac{k_2 [E_0]\ [S]}{\frac{k_{-1} + k_2}{k_1} + [S]} = \frac{k_2 [E_0]\ [S]}{K_m + [S]}. \tag{10}$$

Here $\frac{k_{-1} + k_2}{k_1}$ corresponds to K_m, and it is readily seen that if $k_{-1} >> k_2$, $K_m = \frac{k_{-1}}{k_1} = \frac{1}{K_1}$, which corresponds to the assumption that the first step is in equilibrium. When the condition $k_{-1} >> k_2$ is satisfied, then the determination of K_m from rate curves gives the reciprocal of the equilibrium constant of the first step. Under *these* conditions $1/K_m$ is a measure of the affinity of the enzyme for the substrate. It is noted that the above is the exact equivalent of the Briggs-Haldane formulation of the problem (Haldane, 1930). This case is given here for its illustrative value and for comparison to complex systems. While it is not unreasonable that there should be cases in which $k_2 << k_{-1}$, it should be noted that in the few instances where *individual determinations* of k_1, k_2 and k_{-1} have been made, the results have been quite the converse; i.e., $k_{-1} << k_2$ (Chance, 1943; Goldstein, 1944). In such cases the constant, which would be observed as K_m, would *not* be an equilibrium constant. In the event that $k_2 >> k_1$ and $K_m \sim k_2/k_1$, the constant K_m would exhibit a temperature dependency typical of an equilibrium constant; or, if $\Delta F_2^{\ddagger} \sim \Delta F_1^{\ddagger}$, would exhibit practically no temperature dependency. If $[S] \rightarrow \infty$, from equation (1); $v \rightarrow k_2[E_0]$. Under these conditions $[E] \rightarrow 0$ and $[E \cdot S] \rightarrow [E_0]$. Thus the limiting velocity is the rate of breakdown of $[E \cdot S]$ via step two; this is said to be the limiting step and $[E]$ is said to be the limiting component. In this case, the hyperbolic relation (10) between $[S]$ and v is clearly due to the saturation of the enzyme, and is a direct consequence, analytically, of imposing the material balance (9), because when $[E_0] >> [E \cdot S]$, v is given by equation (8) with $[E] = [E_0]$.

That the existence of a limiting velocity is not necessarily due to the saturation or binding of a given component is seen as follows:

For a system of $n = 3$, equation (3) becomes, if $w_{-3} = 0$,

$$v = \frac{w_1 w_2 w_2}{w_2 w_3 + w_{-1} w_3 + w_{-1} w_{-2}}. \tag{11}$$

If no material balance is necessary, equation (11) gives the correct steady state velocity, and we are assuming that no C_i is in the bound form analogous to $E \cdot S$. From (11) it is seen, recalling the definition of w_i, that v is hyperbolic in $[C_2]$ and $[C_3]$. The limiting velocity is, when $[C_3] \rightarrow \infty$,

$$v = \frac{w_1 w_2}{w_2 + w_{-1}}, \tag{12}$$

which is recognized as the velocity of the first two steps with $w_{-2} = 0$. The interpretation is clear: The value of $[C_3]$ can effect the overall rate of the system only by causing the consumption of X_2 and thus repressing the reversal of step two. From equation (7), when $[C_3] \rightarrow \infty$, $[X_2] \rightarrow 0$. The limiting velocity is the rate at which steps one and two can supply X_2 to step three, w_{-2} being zero. This interpretation is general for such a system and is intimately related to the fact (also pointed out by Burton, 1939) that if any step is made irreversible, the rate becomes independent of the following steps. Thus if the concentration of the kth reactant is increased indefinitely, the rate becomes that of the first $k - 1$ steps. In the case being discussed, no mechanism has been provided for a saturation effect and the limiting velocity cannot be due to a limiting component. Furthermore, in general, no single step becomes limiting; however, if $[C_2] \rightarrow \infty$, v becomes the rate of the first step. It will be noted that v is a linear function of $[C_1]$ and no limiting velocity exists for C_1. It is clear from equation (11) that if $[C_2]$ alone is varied, the quantity which would appear as K_m is

$$\frac{w_{-1}}{k_2} + \frac{w_{-1} w_{-2}}{k_2 w_3},$$

and similarly for $[C_3]$ the apparent K_m is

$$\frac{w_{-1} w_{-2}}{k_3 (w_2 + w_{-1})}.$$

If a component in the system is conserved, the above discussion must be modified. In particular, if a reactant in the first step is regenerated in some subsequent step, its concentration will appear in the w for the reverse of that step. It will then be true, as is evident from equation (3), that a limiting velocity exists for C_1 and C_0. Furthermore, a material balance (4) is necessary for a conserved component. These points will be covered by treating a concrete example.

A Typical Dehydrogenase System.

Consider the system

$$E + S \rightleftarrows E \cdot S$$
$$E \cdot S + Co \rightleftarrows E + Co \cdot H + p$$
$$P + Co \cdot H \rightarrow P \cdot H + Co$$

for which

$$\begin{aligned} w_1 &= k_1 [E] [S] & w_{-1} &= k_{-1} \\ w_2 &= k_2 [Co] & w_{-2} &= k_{-2} [E] [p] \\ w_3 &= k_3 [P] \ ; & w_{-3} &= 0 \ ; \end{aligned} \tag{13}$$

where Co and P are coenzyme and flavoprotein respectively and $Co \cdot H$ and $P \cdot H$ reduced coenzyme and flavoprotein respectively. In general such a system operates by having $P \cdot H$ reoxidized by other "carriers" which are ultimately reoxidized by O_2. This case has been solved, but those steps introduce no new features and are omitted here. The complex $E \cdot S \cdot Co$ has been omitted because its inclusion introduces only additional rate constants. The equations as written amount to the assumption that reduced coenzyme and oxidized substrate leave the E — surface simultaneously.† The expression for v is

$$v = \frac{k_1 k_2 k_3 [E] [S] [Co] [P]}{k_2 k_3 [Co] [P] + k_{-1} k_3 [P] + k_{-1} k_{-2} [E] [p]}. \tag{14}$$

Equation (14) is the correct expression provided $[E]$, the concentration of *free* enzyme, is inserted from

$$\begin{aligned} [E_0] &= [E] + [E \cdot S] \\ [E \cdot S] &= \frac{1}{k_{-1}} \{k_1 [E] [S] - v\} \ ; \end{aligned} \tag{15}$$

for from equation (15), $[E]$ is dependent upon all other concentration terms except when $[S]$ is small. If (14) is written as

$$v = \frac{A [E]}{B + C [E]}, \tag{16}$$

where

$$\left.\begin{aligned} A &= k_1 k_2 k_3 [S] [Co] [P] \ , \\ B &= k_3 [P] (k_2 [Co] + k_{-1}) , \\ C &= k_{-1} k_{-2} [p] \ , \end{aligned}\right\} \tag{17}$$

then

†Strictly speaking, it would be necessary to introduce the possibility of the formation of $E \cdot Co$, $E \cdot Co \cdot H$ and $E \cdot p \cdot Co \cdot H$ with Co, $Co \cdot H$ and S and p characterized by different affinities for the E — surface. If S and Co react sufficiently fast on the E — surface, the concentration of $E \cdot Co$ may be ignored.

$$[E \cdot S] = \frac{1}{k_{-1}} \left\{ \frac{Bk_1 [S] [E] + Ck_1 [S] [E]^2 - A [E]}{B + C [E]} \right\}, \qquad (18)$$

and combining the two members of equation (15) gives

$$[E] = \frac{\left(\frac{A}{k_{-1}} - BK_1 [S] + C [E_0] - B \right)}{2C(1 + K_1 [S])} + \frac{\sqrt{\left(BK_1 [S] - \frac{A}{k_{-1}} + B - C [E_0] \right)^2 + 4B [E_0] C(1 + K_1 [S])}}{2C(1 + K_1 [S])}. \qquad (19)$$

When equation (19) is used in (14) it is seen that v is non-linear in $[S]$ and a limiting velocity will exist when $[S] \rightarrow \infty$. Perhaps more important it should be noted that v is not directly proportional to $[E_0]$, the total enzyme concentration. The non-linearity of equation (14) in $[E]$ is clearly due to the fact that E is regenerated in the second step and the non-linearity of $[S]$ results from the imposed condition (15) which takes into account that E is bound by S in the form $E \cdot S$. The fact that v is proportional to $[E_0]$, a property of the simple system expressed by equation (10), is often assumed to be true for complex systems. That v is *not* directly proportional to $[E_0]$ is a common finding (e.g. Hearon, 1944; Dewan and Green, 1938). As will be evident from the system being discussed, the dependency of v upon $[E_0]$ will be conditioned by the particular steps in which E enters and is regenerated in the system. From equation (19), when $[S] \rightarrow \infty$, $[E] \rightarrow 0$; we will need, in order to examine the limiting velocity in this case, the expression for $\operatorname{Lim}_{[S] \rightarrow \infty} ([E] [S])$. By factoring

$$\left(BK_1 [S] - \frac{A}{k_{-1}} + B - C [E_0] \right)$$

from the radicand and expanding the result, the value of $[E]$ for large $[S]$ is found to be

$$\left. \begin{aligned} [E] &= \frac{B [E_0]}{[S] \{BK_1 - \alpha\}}, \\ \frac{A}{k_{-1}} &= \alpha [S]. \end{aligned} \right\} \qquad (20)$$

The limit sought is thus

$$\lim_{[S]\to\infty} ([E]\ [S]) = \frac{B\ [E_0]}{BK_1 - \alpha} = \frac{B\ [E_0]}{k_1 k_3\ [P]}. \tag{21}$$

Substituting equation (21) into (16), with $[E] = 0$, gives as the limiting velocity when $[S] \to \infty$

$$V_m^{(S)} = k_2\ [Co]\ [E_0]\ . \tag{22}$$

Equation (22) gives v when $[E \cdot S] = E_0$ and $w_{-2} = 0$. This is readily perceived physically since when $[S] \to \infty$, $[E \cdot S] \to [E_0]$ requiring $[E] \to 0$, the second step is rendered irreversible. In an entirely similar manner it may be shown that when $[Co] \to \infty$, $[E] \to [E_0]$ and the limiting velocity is

$$V_m^{(Co)} = k_1\ [S]\ [E_0]\ , \tag{23}$$

which is the *forward* rate of the first step when $[E] = [E_0]$. This is seen to result physically from the fact that when $[Co] \to \infty$, $[E \cdot S] \to 0$ rendering the first step irreversible; the overall rate of v is then independent of the subsequent steps. When $[P] \to \infty$ it can be shown that $[E]$ approaches

$$[E] = \frac{(k_2\ [Co] + k_{-1})\ [E_0]}{k_2\ [Co] + k_1\ [S] + k_{-1}}. \tag{24}$$

Substitution of equation (24) into (14), which for large $[P]$ has the form (25)

$$v = \frac{k_1 k_2\ [E]\ [S]\ [Co]}{k_2\ [Co] + k_{-1}}, \tag{25}$$

gives

$$V_m^{(P)} = \frac{k_2\ [E_0]\ [S]\ [Co]}{\frac{1}{K_1} + [S] + \frac{k_2}{k_1}\ [Co]}, \tag{26}$$

which can be shown to be the rate of the first two steps with $w_{-2} = 0$.

The results (22), (23), and (26) show the extent to which the principles discussed with respect to equation (11) apply to a system in which one or more components are conserved and the modifications which are necessary. The results may be generalized as follows: There will be a V_m for a component occurring in the first step provided that it (1) is conserved in the system and regenerated in a subsequent *reversible* step or (2) is capable of binding the free form of a catalytic component. For any C_i, $i \neq 1$, $i \neq 0$, which does not bind a catalytic component the corresponding $V_m^{(C_i)}$ is the rate of the preceding $i - 1$ steps as already noted. If *any* C_i does bind a catalytic compo-

nent, the corresponding $V_m^{(C_i)}$ is the rate of the subsequent steps $i+1$ through $i+\xi$, terminating irreversibly in the $(i+\xi)$th step, where the free catalyst is regenerated in the $(i+\xi)$th step. In the rate expression for the $(i+1)$th through $(i+\xi)$th steps, the concentration of the bound form of the catalyst in question must obviously be set equal to the total concentration of the catalyst. These general principles, which are difficult to state verbally, may be implemented with the following extension of the case already treated:

(a) $E + S \rightleftarrows E \cdot S$,
(b) $Co + E \cdot S \rightleftarrows E \cdot S \cdot Co$,
(c) $E \cdot S \cdot Co \rightleftarrows E + p + Co \cdot H$,
(d) $Co \cdot H + P \rightarrow P \cdot H + Co$.

Here $E \cdot S \cdot Co$ has been included and its concentration will be regarded as appreciable relative to $[E_0]$. The quantity $V_m^{(S)}$ will be the rate of steps (b) and (c) with $[E \cdot S] = [E_0]$ and $w_{-3} = 0$, since E is regenerated in step (c). The quantity $V_m^{(Co)}$ will be the *forward* rate of step (c) with $[E \cdot S \cdot Co] = [E_0]$. If additional steps were interposed between (c) and (d), these steps would be included in $V_m^{(Co)}$ down through that step regenerating E. The quantity $V_m^{(P)}$ is the rate of steps (a), (b), and (c) with $w_{-3} = 0$. This example includes all of the cases discussed generally above.

These deductions are of importance for the experimental analysis of such systems because they state that the various limiting velocities are given by the rate expressions for a discrete number of steps, the steps are specified and their dependency on the $[C_i]$ given. Although in the case of the dehydrogenase systems, the individual steps or small number of steps may be separately analyzed, the above results indicate that it is not required that such separations be experimentally feasible.

To return to the general solution (14), with $[E]$ from equation (19), it must be noted that v is *not* hyperbolic in $[S]$. However, for large $[S]$, when $[E]^2 << [E]$, the solution for $[E]$ is

$$[E] = \frac{[E_0]\, B}{B + k_1 k_3\, [P]\, [S] - C\, [E_0]}, \tag{27}$$

and equation (27) put into (14) gives v as a rectangular hyperbola in $[S]$.† For still larger $[S]$, v is, of course, $V_m^{(S)}$, and for small $[S]$, $[E] = [E_0]$, and v is linear in $[S]$ and $[E_0]$; at intermediate values of $[S]$ the full expression must be used.

†This result would be formally identical with (10) but with "K_m" $= \dfrac{B}{k_1 k_3 [P]}$.

It is of some interest to inquire under what conditions it may be assumed that the first step, $E + S \rightleftarrows E \cdot S$, of the system be in equilibrium. From equation (19) it is seen that if $C\,[E_0]$ predominates the other terms

$$[E] = \frac{[E_0]}{1 + K_1\,[S]}, \tag{28}$$

which is the equilibrium expression for $[E]$. This is a direct mathematical result and the manner in which the physical requirements may be met is another question. Recalling the definition of A, B, and C in equation (17) and noting that

$$\frac{A}{k_{-1}} - BK_1\,[S] = -k_1k_3\,[P]\,[S],$$

it is seen that equation (28) may be obtained on several bases. For example, if k_2 is large or k_{-1} and k_{-2} are large, if k_3 is small, if $[P]$ is small, etc., (28) is justified. All of the possible conditions have the factor in common that they amount to diminishing those quantities which determine the breakdown of $E \cdot S$ via routes other than dissociation to E and S. Thus the assumptions which justify equation (28) are basically the same as those which make $K_m = \frac{1}{K_1}$. It can also be shown that when $[S]$ is such that $[E] = [E \cdot S] = [E_0]/2$, then $v \neq \frac{V_m^{(S)}}{2}$. In other words,

$$\frac{[E \cdot S]}{[E_0]} \neq \frac{v}{V_m^{(S)}},$$

except when the values are 1 or zero, and the percentage "saturation" of the enzyme does not correspond to the percentage attainment of $V_m^{(S)}$. This is readily shown by considering that, from equation (15)

$$\begin{aligned} v &= w_1\left\{1 - \frac{w_{-1}}{w_1}\,[E \cdot S]\right\} \\ &= w_1\left\{1 - \frac{k_{-1}}{k_1\,[S]}\,\frac{[E \cdot S]}{[E]}\right\}, \end{aligned} \tag{29}$$

and that when $[E] = [E \cdot S] = \frac{[E_0]}{2}$, $[S]$ is found to be, from equation (19)

$$[S] = \frac{B + \frac{C}{2}[E_0]}{K_1\left\{\frac{C}{2}[E_0] + k_{-1}k_3[P]\right\}}. \tag{30}$$

Equation (30) put into (29) gives

$$v = \frac{(1 - K_1^2)\left\{\frac{C}{2}[E_0] + k_{-1}k_3[P]\right\} + k_2k_3[P][Co]}{B + \frac{C}{2}[E_0]}. \tag{31}$$

The ratio of v from equation (31) to $V_m^{(S)}$ from equation (22) clearly depends upon the values of all concentrations except $[S]$ and every k_i. It should be noted that when $[E] = \frac{[E_0]}{2}$ and when equilibrium is assumed in the simple system, the value of $[S]$ is $\frac{1}{K_1}$. From equation (30) it is seen that $[S] = \frac{1}{K_1}$ only if the assumption leading to $C >> B$ can be made; furthermore, from equation (29) the conditions

$$\frac{[E \cdot S]}{[E]} = 1; \qquad [S] = \frac{1}{K_1}$$

are incompatible if $v \neq 0$.

If in the system under discussion the "carrier" P is omitted, the system becomes

$$E + S \rightleftarrows E \cdot S;$$
$$Co + E \cdot S \rightarrow E + p + Co \cdot H;$$

only under the condition that during the time of observation the system is sufficiently far from equilibrium so that $w_{-2} = 0$. The solution is identical with $V_m^{(P)}$ already given in equation (26). This corresponds to a common experimental procedure; viz., in a system of E, S, and Co, the rate of appearance of $Co \cdot H$ is followed optically. E. Negelein and E. Haas (1935) have made an analysis under these conditions of the Zwischerferment – Co_{II} – glucose – 6 – phosphate system. They state that the rate of appearance of $Co_{II} \cdot H$ follows

$$v = R[Co_{II}], \tag{32}$$

and establish empirically the dependency of R upon $[S]$, $[Co_{II}]$ and

$[E_0]$. Comparison of equation (32) with (26) shows that R should be given by

$$R = \frac{k_2 [E_0] [S]}{[S] + \frac{1}{K_1} + \frac{k_2}{k_1} [Co_{II}]}, \tag{33}$$

from which it follows that R is directly proportional to $[E_0]$, a rectangular hyperbola in $[S]$ and R^{-1} a linear function of $[Co_{II}]$. Figures 1 and 2 show R and R^{-1} as functions of $[Co_{II}]$ and R as a function of $[S]$ and $[E_0]$. The correspondence found here is apparently satisfactory. It must be mentioned, however, that from the data it is by no means certain that the conditions of the steady state were fully met; i.e., during the time of observation *appreciable* Co_{II} was con-

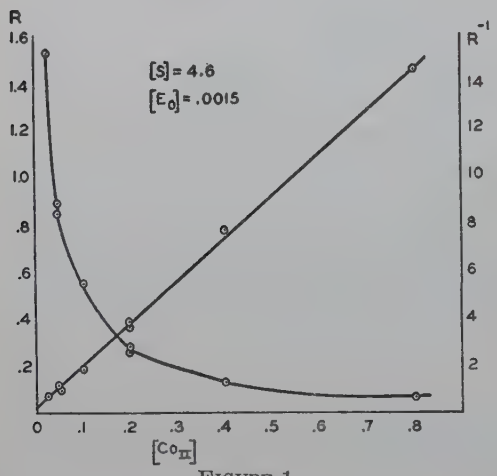

FIGURE 1

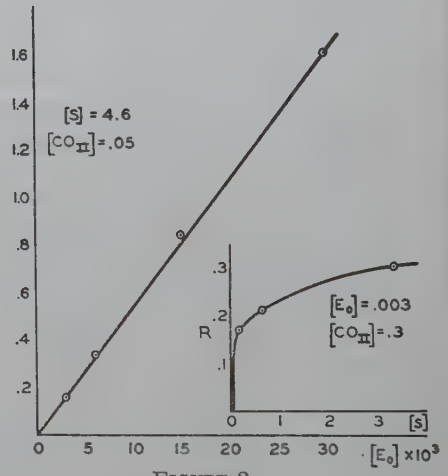

FIGURE 2

verted to $Co_{II} \cdot H$. In equation (33) we are identifying $[Co_{II}]$ with the *total* concentration of Co_{II} which is not strictly true in this case. The assumption that $w_{-2} = 0$ can be fully justified from the data. It might also be mentioned that O. Warburg and E. Negelein (Negelein and Haas, 1935, p. 212) treat the data on the basis that E is in *equilibrium* with Co_{II} and $Co_{II} \cdot H$ and that the equilibrium constant is *identical* for the two species. The rate is then made proportional to $[Co_{II}]$ and $[E_0]$. This analysis gives R in a form which is independent of $[S]$ or, if $[S]$ be included in the proportionality constant, is linear in $[S]$. The data in Figure 2 would seem to make this assumption inadmissable.

The Dilution Effect.

If an enzyme system or tissue brei is diluted and the ratio of the initial and final velocities compared, rather "anamolous" results are obtained. Of course, in the case of tissue preparations, the elution of diffusible components away from components fixed in the large particles and similar effects must be taken into account (Potter, 1941). Since rather unlikely explanations based on termolecular collisions have been given (Krebs, 1942) for the so-called dilution effect, it is of interest to inquire what type of dilution effect would be required by the rate equations being discussed. Taking equation (26) as an illustration, if the system is diluted to twice its original value the ratio of initial and final rates is given by

$$\frac{v_1}{v_2} = 8 \left\{ \frac{\frac{1}{2}\left([S] + \frac{k_2}{k_1}[Co]\right) + \frac{1}{K_1}}{[S] + \frac{k_2}{k_1}[Co] + 1/K_1} \right\}. \tag{34}$$

If

$$[S] + \frac{k_2}{k_1}[Co] >> 1/K_1, \quad v_1/v_2 = 4;$$

if

$$[S] + \frac{k_2}{k_1}[Co] << 1/K_1, \quad v_1/v_2 = 8.$$

In general, $4 \leq v_1/v_2 \leq 8$.

Direct and simple results cannot be obtained for the general solution (14) but it is clear that on the above basis dilution effects approaching 32 can be obtained for a 2-fold dilution of systems involving only four C_i. The ratios obtained by Krebs using the *l*-amino acid oxidase system ranged from 2 to 19. (See for example, Green, 1940.) The dilution effect is of further interest in more general problems. For example, it has been suggested (Potter, 1942) that growth outpaces the syntheses of oxidative enzymes in tumor tissue thereby causing a dilution of these enzymes relative to normal tissue. Of importance also is the subject of the kinetics of the dilution effect; i.e., the time course of transition to a new steady state which will be reserved for forthcoming papers. This problem has been solved for certain cases by A. C. Burton (1939) and discussed by him in connection with "overshoot."

The Turnover Number.

In the system for which equation (14) is the solution, the molecules of *Co* are being "used over again." The ratio

$$\zeta_{Co} = \frac{v}{[Co]} \tag{35}$$

gives the so-called turnover number. ζ_{Co} has the dimensions t^{-1} and is the number of times per unit time a molecule of *Co* is reduced and oxidized. If, for simplicity we assume that the system is operating at large values of $[P]$, then from the equation (26)

$$\zeta_{Co} = \frac{k_2 [S] [E_0]}{[S] + 1/K_1 + \frac{k_2}{k_1} [Co]}, \tag{36}$$

equation (36) gives ζ_{Co} in its dependency on $[S]$, $[E_0]$ and $[Co]$.† The quantity ζ_{Co} exhibits a maximum value of

$$\frac{k_2 [S] E_0}{[S] + 1/k_1}$$

and approaches zero as $[Co] \rightarrow \infty$. In general, ζ for any catalytic component will depend upon the concentration of every other component and values of ζ are not comparable except under strictly comparable conditions (cf. Straus and Goldstein, 1943, p. 580).

Coupled or Branched Systems.

$$\begin{array}{ll} A + B \rightleftarrows X_1 & \\ X_1 \rightleftarrows X_2 + p \longrightarrow & p + E \rightleftarrows E \cdot p \\ X_2 \rightarrow M & E \cdot p \rightarrow E + q\,. \end{array}$$

For the above systems, the velocity of the p–producing system is

$$v_1 = \frac{k_1 k_2 k_3 [A] [B]}{k_2 k_3 + k_{-1} k_3 + k_{-1} k_{-2} [p]}, \tag{37}$$

and that for the p–consuming system is

$$v_2 = \frac{V_m^{(p)} [p]}{K + [p]}; \tag{38}$$

†In an excellent study of this kind (Corran et al, 1939), ζ for Straub's flavoprotein functioning with the lactic-dehydrogenase system has been shown to be a decreasing function of its own concentration as would be predicted from the proper modification of (14).

but the condition determining $[p]$ is

$$k_2 [X_1] - k_{-2} [X_2] [p] = \frac{V_m^{(p)} [p]}{K + [p]}, \tag{39}$$

from which

$$\frac{k_2}{w_{-1}} (w_1 - v_1) - \frac{k_{-2}}{w_2} v_1 [p] = \frac{V_m^{(p)} [p]}{K + [p]}, \tag{40}$$

with the obvious requirement that the left-hand member of equation (40) does not exceed $V_m^{(p)}$. This requires

$$\frac{k_2 w_1}{w_{-1}} \leq V_m^{(p)}. \tag{41}$$

Substituting v_1 from equation (37) into (40) gives for the steady state value of $[p]$

$$[p] = \frac{-a_2^2 - \sqrt{a_2^2 - 4 a_1 a_3}}{2a_1},$$

$$\begin{aligned} -a_2 &= [A] [B] \{k_1 k_3 k_2^2 + k_{-2} k_3 - k_1 k_{-2} k_2\} \\ &\quad + (V_m - k_2 k_1 [A] [B]) \\ a_1 &= k_{-1} k_{-2} (k_2 K_1 [A] [B] - V_m) \leq 0 \\ a_3 &= K (k_2 K_1 - k_2^2 K_1 - k_{-1} k_3) [A] [B]. \end{aligned} \tag{42}$$

Equation (42) put into (37) or (38) gives the rate of either system in terms of the parameters of the other. With the proper modification the above system can be made to correspond to the so-called coenzyme-linked systems (Green, 1940; Dewan and Green, 1937) wherein *Co* is reduced by one enzyme substrate system and reoxidized by another. The net result is the oxidation of the first substrate by the second. This corresponds to the "mutase systems" of D. E. Green et al (1937). One characteristic of such systems is that the rate is not linear in total enzyme concentration. A similar treatment can be made of the true mutases such as aldehyde mutase (Dixon and Lutwak-Mann, 1937). In general p may enter the second system in any step and the solution for this system need not be the simple form (38), but the method of solution remains the same.

Systems Involving Dissociable Prosthetic Groups.

Consider the system

$$\begin{aligned} E + a &\rightleftarrows E^* \\ E^* + S &\rightleftarrows E^* \cdot S \\ E^* \cdot S &\rightarrow E^* + p \end{aligned}$$

in which E is the protein moiety of the enzyme which is active only when associated with the group "a" to form E^*. We wish to examine the steady state solution for this system and to inquire what conditions must be imposed in order that a study of the rate as a function of $[a]$ shall yield the value of the dissociation constant of E^*. Furthermore, the example will introduce a feature which obviates the direct use of equation (2) and its solution (3). Equation (2) will not correctly express the condition $\frac{d\,[E^*]}{dt} = 0$ for $[E^*]$ is reformed in the last step. This situation in which an X_i is regenerated in a subsequent step has not appeared in the cases discussed previously. The steady state condition for $[E^*]$ is

$$\frac{d\,[E^*]}{dt} = k_1\,[E]\,[a] - k_{-1}\,[E^*] - k_2\,[E^*]\,[S] + k_{-2}\,[E^* \cdot S] + k_3\,[E^* \cdot S] = 0\,. \tag{43}$$

Because of the term $k_3\,[E^* \cdot S]$, equation (43) is not equivalent to the corresponding equations which gave rise to (2). Equation (43) must be solved with

$$[E^* \cdot S] = \frac{[E^*]\,[S]}{\frac{k_{-2} + k_3}{k_2}} = \frac{[E^*]\,[S]}{K}, \tag{44}$$

obtained from $\frac{d\,[E^* \cdot S]}{dt} = 0$, subject to the condition

$$[E_0] = [E] + [E^*] + [E^* \cdot S]\,. \tag{45}$$

The solution for $[E^* \cdot S]$ is

$$[E^* \cdot S] = \frac{[E_0]\,[S]\,[a]}{K\,[a] + [S]\,[a] + \frac{K}{K_1}}, \tag{46}$$

and $v = k_3\,[E^* \cdot S]$.

From (46) it is seen that if $[S] << K$, then v is given by

$$v = \frac{k_3\,[E_0]\,[S]\,[a]}{K([a] + 1/K_1)}, \tag{47}$$

and $1/K_1$ may be determined from a study of v as a function of $[a]$. From this it appears that *conditions* may always be arranged such

that (47) is valid regardless of the inherent values of k_1 and k_{-1} (cf. Hogness, 1942).

If the problem is solved on a strict equilibrium basis by solving equations (44) and (45) with the mass action expression (48)

$$[E^*] = K_1 [E] [a] , \tag{48}$$

the solution is identical with (46). This result, which means that the first step may be assumed to be in equilibrium, is general for this type of system. It is seen, in this case, to be a consequence of the requirement

$$\frac{d [E^* \cdot S]}{dt} = 0$$

causing (43) to reduce to (48). If the formation of $E^* \cdot S$ is followed by any number of steps, *any one* of which leads to the restitution of E^* to the system, the requirement that

$$\frac{d [E^* \cdot S]}{dt} = 0$$

and $\frac{d [X_i]}{dt} = 0$ for any $[X_i]$ involved in these steps will reduce the equation corresponding to (43), to (48). For examples, the use of studies of v as a function of $[a]$ to determine K_1 where the equivalent of (47) is assumed to hold, the reader is referred to T. R. Hogness (1942) and F. Lipmann (1939).

Another system which must be treated in the manner here demonstrated is

$$\begin{aligned} E_i &\rightleftarrows E_a \\ E_a &\rightleftarrows E_a \cdot S \\ E_a \cdot S &\rightarrow E_a \end{aligned}$$

where E_i and E_a are inactive and active forms of the enzyme. The solution may be obtained from equation (46) by setting $[a] = 1$. Strictly, one should include the possibility that $E_i \cdot S$ is formed, but this condition is easily included in (45). More generally the above system would consist of a complex chain following the formation of E_a from E_i, equation (44) would then be replaced by the proper form of (5) or (6) and the general method of solution is invarient.

An additional variation would be that the substrate, rather than the enzyme, be elaborated from a precursor. The more general problem is the case in which the substrate (or any C_i) is furnished to the system proper by some process, physical or chemical. This problem will be discussed in a later paper.

The individual cases treated in this paper include, with some obvious and clearly indicated extensions, the majority of the types of systems encountered in cellular respiration. The aerobic dehydrogenases which require either Co_I or Co_{II} would be included in the "typical dehydrogenase system" by merely including the steps which lead to the reoxidation of reduced flavoprotein. The diphosphothiamine enzymes and the autoxidizable flavoprotein enzymes would be included in the "systems involving dissociable prosthetic groups." In particular, for the Lipmann system (Lipmann, 1939) and similar systems, one must include the association of the enzyme with Mg^{++} and replace the substrate, S, by the adjunct between the substrate and phosphate. This latter feature may be included in the problem by considering the addition compound between pyruvate and phosphate to be in equilibrium with pyruvate and inorganic phosphate. For the flavoprotein enzymes which are autoxidizable, e.g., d-amino acid oxidase, it is only necessary to specify that $E^* \cdot S$ react to give $E^* \cdot H_2$ and product, where $E^* \cdot H_2$ is the enzyme-reduced prosthetic group complex, and to include the reoxidation of $E^* \cdot H_2$ by O_2. The steady state condition for $[E^*]$ is still given by (43) with k_3 $[E^* \cdot S]$ replaced by k_4 $[O_2]$ $[E^* \cdot H_2]$. As previously mentioned the "coupled or branched system" can be easily made to correspond to the coenzyme-linked systems. The coenzyme-linked dehydrogenases may in turn be part of more extensive systems as, for example, in the CO_2 fixation reactions leading to the synthesis of the tricarboxylic acids (Ochoa, 1948). It should be noted that the "coupled or branched system" chosen here as an example is so constituted that the parameters of the second, (p-consuming), system *control* the overall rate of the first system. If the second system consumed the product produced in the *last* step of the first system, the two systems would simply constitute a single steady state system and could be treated as such. This example is thus a *branched* system in the sense that two products formed in a given step suffer subsequent fates via different routes. A notable example of such a case is that of the metabolic reactions which generate energy-rich phosphate compounds and whose rates depend upon the phosphate acceptor systems with which they are coupled in a branched manner (for discussion see Lipmann, 1941).

Finally, it should be noted that the general formulation used here is applicable to *cyclic* systems which occupy an important position in the present-day concept of intermediary metabolism. If the product B_n is identical with one of the reactants, C_0 or C_1, in the first step, it re-enters the system. The system is then cyclic. No new features are introduced. The condition that the time rate of change of this component be zero is identical with the equality of the 1st and nth

members of the set of equations (2). This feature, in modified form, has in fact already been encountered in the "typical dehydrogenase system" wherein the enzyme, E, which enters in the 1st step is regenerated in the 2nd step. This is an excellent example of the maintenance of the steady state concentration of a component which is conserved to the system. The requirement that $[E]$ be maintained constant in *this* manner is not a mathematical overstatement of the problem, for this requirement is equivalent to the equality of the *net* rates of the 1st and 2nd steps. In general, this requirement will be equivalent to the equality of some two members of the set of equations (2). The distinction between this physical situation and that giving rise to equation (43) should be carefully noted.

LITERATURE

Burton, A. C. 1939. "The Properties of the Steady State Compared to Those of Equilibrium in Characteristic Biological Behavior." *Jour. Cell. and Comp. Physiol.*, **14**, 327.

Chance, B. 1943. "The Kinetics of the Enzyme-Substrate Compound of Peroxidase." *Jour. Biol. Chem.*, **151**, 553.

Christiansen, J. H. 1935. "Einige Bemerkungen zur Anwendung der Bodensteinschen Method der Stationaren Konzentrationen." *Z. Physik. Chem.*, **28B**, 303.

Corran, J. G., Green, D. E., Straub, F. E. 1939. "On the Catalytic Function of Heart Flavoprotein." *Biochem. Jour.*, **33**, 793.

Dewan, J. G. and Green, D. E. 1937. "Coenzyme-Linked Reactions Between Dehydrogenase Syctems." *Biochem. Jour.*, **31**, 1074.

Dewan, J. G. and Green, D. E. 1938. "Coenzyme Factor — A New Oxidation Catalyst." *Biochem. Jour.*, **32**, 626.

Dixon, M. and Lutwak-Mann, C. 1937. "Aldehyde Mutase." *Biochem. Jour.*, **31**, 1362.

Green, D. E. 1940. *Mechanisms of Biological Oxidations.* Cambridge: The University Press.

Green, D. E., Needham, D., and Dewan, J. 1937. "Dismutations and Oxidoreductions." *Biochem. Jour.*, **31**, 2327.

Goldstein, A. 1944. "The Mechanism of Enzyme-Substrate-Inhibitor Reactions." *Jour. Gen. Physiol.*, **27**, 527.

Hammett, L P. 1937. *Physical Organic Chemistry.* New York: McGraw-Hill.

Haldane, J. B. S. 1930. *Enzymes.* New York: Longmans, Green & Co.

Hearon, J. Z. 1944. "Components of the Glucose Dehydrogenase System." *Thesis.* University of Michigan.

Hearon, J. Z. 1948. "The Kinetics of Blood Coagulation." *Bull. Math. Biophysics*, **10**, 175.

Hogness, T. R. 1942. "The Flavoproteins." *A Symposium on Respiratory Enzymes.* Madison: University of Wisconsin Press.

Krebs, H. A. 1943. "The Intermediary Stages in the Biological Oxidation of Carbohydrate." *Adv. Enzymology*, **3**, 191.

Lipmann, F. 1939. "An Analysis of the Pyruvic Acid Oxidation System." *Symp. Quan. Biol.*, **7**, 248.

Lipman, F. 1941. "Metabolic Generation and Utilization of Phosphate Bond Energy." *Adv. Enzymology*, **1**, 99.

Michaelis, L., and Menton, L. 1913. "Die Kinetik der Invertinwirkung." *Biochem. Z.*, **49**, 333.

Morales, M. F. 1947. "A Note on Limiting Reactions and Temperature Coefficients." *Jour. Cell. and Comp. Physiol.*, **30**, 303.

Negelein, E., and Haas, E. 1935. "Uber die Wirkungsweise des Zwischenferments." *Biochem. Z.*, **282**, 206.

Ochoa, S. 1948. "Biosynthesis of Tricarboxylic Acids by CO_2 Fixation (III) Enzymatic Mechanisms." *Jour. Biol. Chem.*, **174**, 133.

Potter, V. 1941. "Studies on the Mechanism of Hydrogen Transport in Animal Tissues." *Jour. Biol. Chem.*, **141**, 775.

Potter, V. 1942. "Phosphorylation Theories and Tumor Metabolism." *A Symposium on Respiratory Enzymes.* Madison: University of Wisconsin Press.

Straus, O. H., and Goldstein, A. 1943. "Zone Behavior of Enzymes." *Jour. Gen. Physiol.*, **26**, 559.

BULLETIN OF
MATHEMATICAL BIOPHYSICS
VOLUME 11, 1949

THE GROUP STRUCTURE OF SOME NEURAL NETS

J. B. ROBERTS
COMMITTEE ON MATHEMATICAL BIOLOGY
THE UNIVERSITY OF CHICAGO

A method is given for using a set of disjoint cycles as the main body of a neural net that will give rise to more than one temporal response pattern for different afferent stimuli. The method arises out of considering the correspondence between this type of net and certain Abelian groups of finite order. The consideration also gives rise to a possible definition of the "complexity" of this type of neural net.

We consider here a class of neural nets consisting of a set, $\{A_i\}$, of afferents; a set of k disjoint cycles, $\{X_i\}$, with $n_1, n_2, \cdots, n_k$ synapses respectively with the afferents; a set of internuncials connected to the cycles, but not members of them, $\{I_i\}$; and lastly a set of efferents, $\{E_i\}$, see Figure 1.

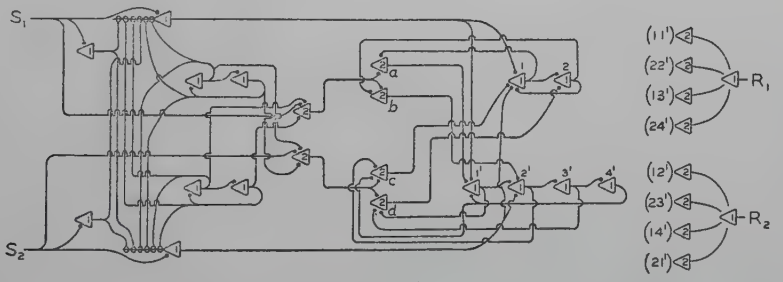

FIGURE 1

In the following we will use the individual cycles as our units of structure of any such net. We label the cycles $1, 2, \cdots, k$. We designate the fact that the $j+1$st synapse in the ith cycle receives an impulse at the time $t+j+1$ by the expression

$$\alpha_i(t+j) = ({}_i\alpha_{j+1}, {}_i\alpha_{j+2}, \cdots, {}_i\alpha_{n_i}, {}_i\alpha_1, \cdots, {}_i\alpha_j). \qquad (1)$$

In the right-hand side of expression (1) we have the ${}_i\alpha_{j+l}$ as the labels of the synapses in the ith cycle. We will designate by t_0 a moment when the only activity in the net is a set of k impulses, one in each of the k cycles. In general we can consider this t_0 as the time at

which the circuit has reached a steady state activity. For the present we consider only the case where one pulse only is present in any given cycle at any time. Also, for convenience, we will consider the synapse in the jth cycle which receives an impulse at t_0 as being labeled ${}_j\alpha_1$. We now define a power of $\alpha_i(t_0)$ by

$$\alpha_i^{j+1}(t_0) = {}_{Df}\alpha_i(t_0 + j). \tag{2}$$

In addition, we define the product of two powers by

$$\alpha_i^{k_1}(t_0) \cdot \alpha_i^{k_2}(t_0) = {}_{Df}\alpha_i^{k_1+k_2}(t_0). \tag{3}$$

From expressions (1), (2), and (3) it follows that

$$\alpha_i(t+q) \cdot q \equiv p\,(mod\, n_i) \cdot \rightarrow \cdot \alpha_i(t+p) = \alpha_i(t+q). \tag{4}$$

We shall also introduce the following notation

$$I_{i_1,\cdots,i_l} = {}_{Df}\frac{\overset{k}{\underset{j=1}{\Pi}}\alpha_j^{n_j}(t_0)}{\underset{m=i_1}{\overset{i_l}{\Pi}}\alpha_m^{n_m}(t_0)}; \tag{5}$$

$$\left.\begin{aligned} B_i(t_0+l) &= {}_{Df}\alpha_i(t_0+l) \cdot I_i \\ &= {}_{Df}B_i^{l+1}(t_0); \end{aligned}\right\} \tag{6}$$

$$G_i = {}_{Df}\{B_i(t_0), B_i^2(t_0), \cdots, B_i^{n_i}(t_0)\}; \tag{7}$$

$$C_{i_1,\cdots,i_l} = {}_{Df}B_{i_1}^1(t_0) \cdot B_{i_2}^1(t_0) \cdots B_{i_l}^1(t_0); \tag{8}$$

$$\begin{aligned} G_{i_1,\cdots,i_l} = {}_{Df}\{&C_{i_1,\cdots,i_l}, C_{i_1}^2,\cdots,i_l, \\ &\cdots, C_{i_1}^L,\cdots,i_l\}; \end{aligned} \tag{9}$$

where $L = LCM\ (n_1, \cdots, n_k)$. We shall refer to expression (9) as the "actual product" of $G_{i_1}, G_{i_2}, \cdots, G_{i_l}$. We distinguish from this "actual product" another product that we call the "formal product";

$$\begin{aligned} B_i^{l_1}(t_0)\,\varepsilon\, G_i \cdot B_j^{l_2}(t_0)\,\varepsilon\, G_j \rightarrow \bar{G}_{i,j} = {}_{Df}G_i \times G_j = {}_{Df}\{&B_i^{l_1}(t_0) \\ &\cdot B_i^{l_2}(t_0)\}. \end{aligned} \tag{10}$$

Theorem 1. *The sets defined in equations* (7) *and* (9) *are multiplicative, cyclic, groups of orders* n_i *and LCM* $(n_{i_1}, \cdots, n_{i_1})$ *respectively.*

Proof. As both are finite sets we need only show closure and commutativity under multiplication. We have

$$B_i^j(t_0) \cdot B_i^m(t_0) = B_i^{j+m}(t_0) = B_i^{m+j}(t_0) = B_i^m(t_0) \cdot B_i^j(t_0),$$

from equations (2), (3), (4), and (6). The identity of the group is $B_i^{n_i}(t_0)$. This shows that the set (7) satisfies the theorem. The proof for the set (9) is almost identical. It will be noted that the set (7) is included in the set (9). The total number of groups of the form of set (9) obtainable from our k disjoint cycles is equal to 2^k.

The product defined in (10) can now be seen to be a direct product since we have all k cycles disjoint from one another. We now consider the general product

$$G_{i_1} \times G_{i_2} \times \cdots \times G_{i_l} = \bar{G}_{i_1, \cdots, i_l}. \tag{11}$$

Theorem 2. *The formal product of* l *of the* k *cycles as defined in equation* (11) *is an Abelian group of order* $n_{i_1}, \cdots, n_{i_l}$.

Proof. $\bar{G}_{i_1, \cdots, i_l}$ is the product of l cyclic groups all of which have the same identity ((5), (6), (7), (8), (9)). Each of the cyclic groups is disjoint from the others except for the identity; hence, their product is direct and composes a group $\bar{G}_{i_1, \cdots, i_l}$. This group is Abelian and its order is the product of $n_{i_1}, \cdots, n_{i_l}$.

Corollary 1. The groups of Theorem 1 are all subgroups of the general group $\bar{G}_{1, 2, \cdots, k}$.

Corollary 2. The order of $\bar{G}_{1, 2, \cdots, k}$ may have divisors that are not orders of any of the groups of Theorem 1. Suppose, for example, that the smallest order of the groups of Theorem 1 is $2q$. Then the divisor 2 of the order of $\bar{G}_{1, \cdots, k}$ is not the order of any subgroup of Theorem 1.

Corollary 3. All divisors of the order of $\bar{G}_{1, 2, \cdots, k}$ are orders of subgroups of $\bar{G}_{1, \cdots, k}$ that are contained in Theorem 1 if, and only if, the following condition holds: All of the n_i are primes or prime powers such that with any prime power all other smaller positive powers are represented among the n_i.

Proof. (a) If the n_i are all primes then the only divisors of $n_1 \cdots n_k$ are the primes themselves, or some product of them. All such products are represented as orders in the groups of Theorem 1. (b) If some $n_m = p^q$ and p^{q-1}, p^{q-2}, $\cdots$, p are not included as the values of some of the other n_i's, then the ones that are missing represent divisors that are not orders of the groups of Theorem 1. In all cases other than (a) and (b) of the corollary, we will have such divisors.

So far we have seen that all groups included in Theorem 1 are

subgroups of $\vec{G}_{1,2,\cdots,k}$. The largest subgroup of $\bar{G}_{1,\cdots,k}$ included in Theorem 1 is the group $G_{1,2,\cdots,k}$ with index equal to

$$\frac{n_1 \cdots n_k}{\text{LCM } (n_1, \cdots, n_k)} = \text{HCNF } (n_1, \cdots, n_k) = H_1. \tag{12}$$

HCNF $(n_1, \cdots, n_k)$ was defined previously (Roberts, 1948) by

$$\text{HCF} \prod_{i=1}^{k} \left(\prod_{j=1}^{k} n_j/n_i \right).$$

We shall denote by a pathway the progressive excitation of sets of k synapses (one from each cycle) from some time t to the time $t + p$, where p is the period of the set of cycles. Thus a pathway is the totality of simultaneously acting sets of synapses, k-tuples, that can arise through a single impulse acting in all k cycles. By disjoint pathways (*dp*'s) we shall mean pathways which have no k-tuples in common.

Theorem 3. *If* $G_{i_1, \cdots, i_l}$ *has index* q *in* $\bar{G}_{i_1, \cdots, i_l}$, *then there are* q dp's *through the* l *cycles* $i_1, \cdots, i_l$.

To prove this theorem and to find the q *dp*'s one needs only to find the $q - 1$ cosets of $G_{i_1, \cdots, i_l}$ in $\bar{G}_{i_1, \cdots, i_l}$. Each coset and the subgroup itself constitute *dp*'s. The elements of the cosets are the k-tuples of the pathways.

Characterization Matrices. In some instances the cosets of $G_{i_1, \cdots, i_l}$ in $\bar{G}_{i_1, \cdots, i_l}$ are not too difficult to find. We give here a method that depends on a matrix representation of $G_{i_1, \cdots, i_l}$. The matrix (a) of expression (13) is the matrix for $G_{i_1, \cdots, i_l}$.

$$
\begin{array}{cccc}
1 & 1 & \cdots & 1 \\
2 & 2 & \cdots & 2 \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
n_{i_1} & n_{i_1} & \cdots & n_{i_1} \\
1 & \cdot & \cdot & \cdot \\
2 & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & n_{i_2} & \cdots & n_{i_2} \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
n_{i_1} & n_{i_2} & \cdots & n_{i_l}
\end{array}
\qquad \text{(a)}
$$

$$
\left[\begin{array}{cccc}
1 & 1 & \cdots & 1 \\
2 & 2 & \cdots & 2 \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
d-1 & d-1 & \cdots & d-1 \\
0 & 0 & \cdots & 0 \\
1 & 1 & \cdots & 1 \\
2 & 2 & \cdots & 2 \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
\cdot & \cdot & \cdot & \cdot \\
0 & 0 & \cdots & 0
\end{array}\right]
\qquad \text{(b)}
\qquad (13)
$$

If we now reduce all elements of the matrix (a) in expression (13) by d, where $d = \text{HCF}\ (n_{i_1}, \cdots, n_{i_l})$, we get (b). The bracketed part of matrix (b) in expression (13) we call the "characterization matrix" (CM) of (a) or of $G_{i_1}, \cdots, {}_{i_l}$. (If $d = 1$, then the CM can be thought of as being (a) itself. We interchange (a) and $G_{i_1}, \cdots, {}_{i_l}$ in our discussion as if they were identical.) If we now define the operation of cyclically permuting a column by one unit, that is, changing the column $\{1, 2, \cdots, d-1, 0\}$ to $\{2, \cdots, d-1, 0, 1\}$ and then cyclically permute an arbitrary number of columns by any number of units, we shall get a maximum of d^l different rows (the number of ways d different kinds of things can be put into l boxes). As there are d rows in the CM these d^l different rows can make up d^{l-1} matrices of d rows each such that all column sums are $1 + 2 + \cdots + d - 1$. The original CM is one of these d^{l-1} matrices and the others are corresponding CM's for the cosets of $G_{i_1}, \cdots, {}_{i_l}$. For each CM it is clear that we can return to a complete matrix (a) such that no two CM's give matrices (a) with an identical row. For every (a) there is one, and only one, CM possible. However the converse is not true.

As we have

$$\frac{n_{i_1} \cdots n_{i_l}}{\mathrm{LCM}\,(n_{i_1}, \cdots, n_{i_l})} = H_1,$$

we immediately see that $d^{l-1} | H_1$. Now we see that if $d^{l-1} = H_1$, there is a 1 — 1 correspondence between the cosets of $G_{i_1}, \cdots, {}_{i_l}$ and the CM's obtained above. In this case the cosets are completely determined by the CM's. If, however, $d^{l-1} = \frac{1}{q} H_1$ (q an integer) we have a one-many correspondence between the CM's and the cosets of $G_{i_1}, \cdots, {}_{i_l}$. In this case the method finds $\frac{H_1}{q}$ of the cosets uniquely, but the rest have to be found by trial.

One can also merely cyclically permute the columns of (a) to get all of the coset representations (as one has to do when $d = 1$), but this is a more difficult task than the method of the CM's given above, especially when l becomes large. Note that when d^{l-1} is equal to H_1 this means that the numbers $n'_{i_1}, \cdots, n'_{i_l}$, obtained from $n_{i_1}, \cdots, n_{i_l}$ under division by d, are relatively prime in pairs.

We have seen that two disjoint pathways are two sets of k-tuples of synapses such that no k-tuple is common to both sets. In some cases such disjoint pathways can be seen to give rise to other pathways disjoint with them. As an example, consider the k-tuples $(1_1, 1_2, \cdots, 1_k)$ and $(1_1, 1_2, \cdots, 1_{k-2}, 2_{k-1}, 2_k)$ which are members of the *dp*'s A_1, A_2 respectively and which are both acting at time t. From these two we see that the k-tuple $(1_1, 1_2, \cdots, 1_{k-1}, 2_k)$ is also acting at time t and gives rise to another *dp* disjoint from A_1 and A_2. When such is the case we shall say that the *dp*'s A_1 and A_2 are not "effectively disjoint pathways" (*edp*'s).

In other words, any k-tuple formed in the above manner from the k-tuples of a set of *edp*'s is already a member of one of the *edp*'s of the set.

We denote by the *set of effectively disjoint pathways* through a set of k cycles the maximum number of *dp*'s that can exist together simultaneously in a net without giving rise to other *dp*'s. There is a unique correspondence between a set of D afferents and a set of D efferents of the net when the net contains D *edp*'s. We see that the number of *edp*'s is not necessarily the same as the number of *dp*'s.

If we define the quantity H_2 as follows:

$$H_2 = \mathrm{HCF} \prod_{\substack{i,k=1 \\ i \neq k}}^{k} \left(\prod_{j=1}^{k} n_j / n_i \cdot n_j \right), \tag{14}$$

the following three relations hold between the number, D, of *edp*'s and the quantities H_1 and H_2 and LCM $(n_1, \cdots, n_k)$.

$$\begin{aligned} &\mathrm{HCF}(n_1, \cdots, n_k) \leq D \leq H_1, \\ &H_2 = 1 \rightarrow D = H_1, \\ &\mathrm{LCM}(n_1, \cdots, n_k) = \mathrm{LCM}(n_1, \cdots, n_{k-1}) = n_k \rightarrow D \geq n_k. \end{aligned} \tag{15}$$

The exact determination of the value of D in the general case has not yet been made.

From the above considerations we see that if we define "complexity" of a net to be the number of *edp*'s of the net, then the HCNF of the numbers of synapses in the disjoint cycles of the net will be an upper bound of the complexity and can be used to compare two nets, in a limited sense. The actual measure, D, of the complexity under this definition is, so far, an undetermined function of the n_i's. If a net has a number of *edp*'s equal to D, then we can put D afferents into 1 — 1 correspondence with D efferents through the set of k cycles of the net, with each afferent giving rise to a temporal response pattern of any form whose period is $\leq \mathrm{LCM}(n_1, \cdots, n_k) = P$ and which has for the maximum number of neurons in a cross section taken through the cycles of the net

$$2k + \sum_{i=1}^{k} n_i \tag{16}$$

neurons. With the exception of a few isolated cases this method does require the q afferent stimuli to be in "phase" before impinging on the cycles. This will cause a time delay to be introduced between the q afferent neurons and the k cycles.

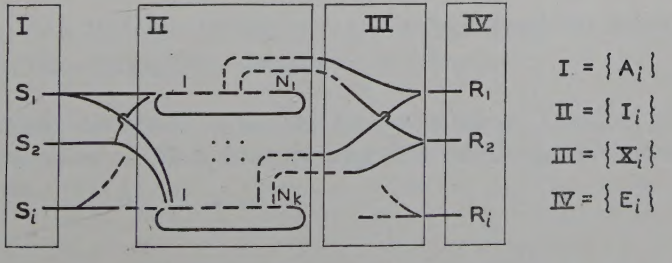

FIGURE 2

As an example of a simple circuit where we use the same two cycles for the passage of two impulses we give Figure 2 for $G_{2,4}$. The numbers in parentheses in the figure indicate the synapses in the two cycles respectively which send neurons to that synapse.

Comparing the present results to those of the previous paper (Roberts, 1948), we see that to each element $C^m{}_{i_1, \cdots, i_l} \varepsilon G_{i_1, \cdots, i_l}$ there corresponds a set of synapses, one from each of the l cycles considered, all acting at the time $t_0 + m$. Also the "range" of the net consisting of just these l cycles is equal to the order of $G_{i_1, \cdots, i_l}$.

This work was aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

LITERATURE

McCulloch, W. S. and Pitts, W. 1943. "A Logical Calculus of the Ideas Immanent in Nervous Activity." *Bull. Math. Biophysics*, 5, 115–133.

Roberts, J. B. 1948. "Mathematical Biophysics of Some Neural Nets." *Bull. Math. Biophysics*, 10, 1–10.